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TITLE OF THE INVENTION (280 characters max)

CAB MOLECULES

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Respectfully submitted,

SIGNATURE _____ Date: December 12, 2003

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Date: December 12, 2003 By: Kay L. Gaviglio

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CAB MOLECULES

FIELD OF THE INVENTION

The present invention relates to CAB molecules, ADEPT constructs directed against CEA, and their use in diagnosis and therapy.

BACKGROUND

Traditional therapeutic molecules circulate freely throughout the body of patients, until they are removed from circulation by the liver or another mechanism of clearance. Such non-targeted molecules exert their pharmocological effects indiscriminately on a wide range of cells and tissues. This can cause serious side effects in the patient. The problem is particularly acute when the molecule is a highly toxic chemotherapeutic agent used to kill cancer cells where the therapeutic window, that is the difference between an efficacious dose and an injurious, or even lethal, dose can be small. Thus, in recent years, researchers have attempted to develop compounds that specifically affect particular subsets of cells, tissues or organs in a patient. Most of the compounds target a particular tissue by preferentially binding a particular target molecule displayed by the tissue to be treated. By preferentially affecting targeted cells, tissues or organs, the difference between an efficacious dose and an injurious dose can be increased, which in turn increases the opportunity for a successful treatment regimen and reduces the occurrence of side effects.

One version of an approach that utilizes preferential binding is antibody-directed enzyme prodrug therapy (ADEPT). *See, e.g.*, Xu *et al.*, 2001, Clin Cancer Res. 7:3314-24.; Denny, 2001, Eur J Med Chem. 36:577-95. In ADEPT, an antibody or antibody fragment is linked to an enzyme capable of converting a pro-drug into an active cytotoxic agent. The ADEPT conjugate is administered to the patient, and the conjugate

is localized to a target tissue. The prodrug is then subsequently administered to the patient. The prodrug circulates throughout the body of the patient, but causes few or no side effects because it is in its inactive form. The prodrug is converted into its active drug form by the localized ADEPT conjugate's enzyme. Because the ADEPT conjugate is localized to the target tissue, the prodrug is activated only in the vicinity of the target tissue. Thus, a relatively low concentration of active drug is present throughout the body, but a relatively high concentration of active drug is produced in the vicinity of the target tissue, allowing the drug to exert its therapeutic effects at the desired site, increasing the therapeutic window of the toxin.

Carcinoembryonic antigen ("CEA") was first described by Gold and Freedman, J. Exp. Med., 121, 439-462, (1965). CEA is expressed by most colorectal cancers and by a number of other tumors. CEA is highly expressed in tumor tissue, and it is also found at a lower concentration in normal organs in particularly in the digestive tract.

SUMMARY OF THE INVENTION

The present invention relates to CAB molecules, ADEPT constructs directed against CEA, and their use in diagnosis and therapy.

In a first aspect, the invention is drawn to a CAB molecule comprising a modified amino acid sequence. In one embodiment, the CAB molecule has an amino acid sequence modified from the amino acid sequence set forth in SEQ ID NO:1, and the modification is at least one position selected from the group consisting of positions 100, 102, 104, 105, 107, 163, 165, 166, 184 and 226, wherein position numbering is with respect to SEQ ID NO:1 as shown in Figure 1. In a preferred embodiment, the CAB molecule comprises modifications at positions 100, 184 and 226. In a preferred embodiment, the CAB molecule comprises modifications at positions 100, 102, 104, 105, 107, 163, 165, 166, 184 and 226.

In a preferred embodiment, the modification is at least one selected from the group consisting of T100L, T102L, P104A, Y105I, F107N, S163A, S165Y, Y166A, S184D and S226D, wherein position numbering is with respect to SEQ ID NO:1 as

shown in Figure 1. In a preferred embodiment, the CAB molecule comprises a CAB1.6 molecule, the CAB1.6 molecule having the following modifications: T100L, S184D and S226D. In a preferred embodiment, the CAB molecule comprises a CAB1.7 molecule, the CAB1.7 molecule having the following modifications: T100L, T102L, P104A, Y105I, F107N, S163A, S165Y, Y166A, S184D and S226D.

In a preferred embodiment, the CAB molecule comprises the scFV portion of CAB1.6 (SEQ ID NO:5), or CAB1.7 (SEQ ID NO:6).

In a preferred embodiment, the CAB molecule further comprises a beta-lactamase molecule. In a preferred embodiment, the CAB molecule has an amino acid sequence modified from the amino acid sequence set forth in SEQ ID NO:2, and the modification is at least one position selected from the group consisting of positions: 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184, 226, 265 and 568, wherein position numbering is with respect to SEQ ID NO:2 as shown in Figure 2. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 146, 181, 184 and 226. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184 and 226. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184, 226, 265 and 568.

In a preferred embodiment, the CAB molecule has modifications comprising at least one modification selected from the group consisting of K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D, S226D, K265A and S568A, wherein position numbering is with respect to SEQ ID NO:2 as shown in Figure 2. In a preferred embodiment, the CAB molecule comprises a CAB1.6 molecule, the CAB1.6 molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, M146V, W181V, S184D and S226D. In a preferred embodiment, the CAB molecule comprises a CAB1.6i molecule, the CAB1.6i molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, M146V, W181V, S184D, S226D, K265A and S568A. In a preferred embodiment, the CAB molecule comprises a CAB1.7 molecule, the CAB1.7 molecule comprising the following

modifications: K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D and S226D. In a preferred embodiment, the CAB comprises a CAB1.7i molecule, the CAB1.7i molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D, S226D, K265A and S568A.

In a preferred embodiment, the CAB molecule comprises CAB1.6 (SEQ ID NO:7), CAB1.6i (SEQ ID NO:8), CAB1.7 (SEQ ID NO:9) or CAB1.7i (SEQ ID NO:10).

In a second aspect, the invention is drawn to a nucleic acid encoding a CAB molecule as set forth herein. In a third aspect, the invention is drawn to treating a subject in need thereof, comprising administering to the subject a CAB molecule, as provided herein, and a prodrug that is a substrate of the CAB molecule. In a fourth aspect, the invention is drawn to a pharmaceutical composition comprising a CAB molecule.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 sets forth the amino acid sequence of the six CDRs of an unmodified CAB molecule. Position numbering starts with the first position of H1, as set forth in SEQ ID NO:2 as shown in Figure 2A. Position numbering of the 6 CDRs with respect to SEQ ID NO:1, as shown in Figure 1, is as follows: H1:26-35; H2, 50-65; H3, 99-109; L1, 159-168; L2, 184-190 and L3, 223-231.

Figure 2 sets forth the amino acid sequence of the CAB1 molecule (2A) and the amino acid sequence for BLA (2B).

Figure 3 sets forth the amino acid for the CAB1.6 CDR (3A) and the CAB1.7CDR (3B).

Figure 4 sets forth the amino acid sequence for the CAB1.6 (4A) and CAB 1.6i (4B) molecule.

Figure 5 sets forth the amino acid sequence for the CAB1.7 (5A) and CAB1.7 i (5B) molecule.

Figure 6 present details related to plasmid pME27.1. Figure 6A presents a schematic diagram of plasmid pME27.1. P lac = lac promoter, Pel B leader sequence =

signal seq, CAB1 scFv=single chain antibody, BLA= β-lactamase gene, CAT = chloramphenicol acetyl transferase resistance gene, T7 terminator=terminator. Figure 6B shows the sequence of CAB1-scFv, the CDRs and mutations chosen for combinatorial mutagenesis. Figure 6C presents and nucleotide sequence of pME27.1. Figure 6D shows the amino acid sequence of CAB1 that shows, for example, the sequence of the heavy chain, the sequence of the linker, the sequence of the light chain and the sequence of BLA.

Figure 7 shows binding assays and SDS PAGE (polyacrylamide gel electrophoresis results. Specifically, Figure 7A shows the binding of variants from library NA05; Figure 7B displays and SDS PAGE of stable CAB1-BLA variants of the NA05 library; Figure 7C shows binding of various isolates from NA06 to CEA.

Figure 8 shows a comparison of vH and vL sequences of CAB1-scFv with a published frequency analysis of human antibodies. Specifically, Figure 8A shows the observed frequencies of the five most abundant amino acids in alignment with the human sequence in the heavy chain; Figure 8B shows the observed frequencies of the five most abundant amino acids in alignment with the human sequence in the light chain.

Figure 9 shows screening results of NA08 library. The x-axis shows binding at pH 7.4, and the Y-axis shows binding at pH 6.5. Clones that were chosen for further analysis are represented by a square.

Figure 10 shows a three dimensional model with positions that were chosen for combinatorial mutagenesis.

Figure 11 shows pH-dependent binding of NA08 variants to immobilized CEA. The x-axis shows BLA activity, and the Y-axis shows CEA bound activity. Variant designations are shown in the top left corner.

Figure 12 sets forth a CAB engineering summary. The left column refers to the protein designation. The middle column details cumulative changes from the previous line. The right column provides a putative reason for each of the mutations, as provided in the text of the document. For example, changes were made from CAB1 to CAB1.1 to increase the overall stability of the protein, as provided herein. As can be seen from the

column, changes were also made to increase, among other things, the pH-dependent binding of a molecule, increase affinity and remove T-cell epitopes.

Figure 13 sets forth binding of various CAB1 variants to immobilized CEA. Binding to CEA (x-axis) and BLA activity (y-axis) show, for example, different characteristics at different binding pHs.

Figure 14 sets forth binding of various CAB1 variants to LS174T cells. Binding characteristics are shown for LS174T cells, the protocol as described herein. Again, different binding characteristics can be seen at different pHs.

Figure 15 discloses relevant sequences as follows: Figure 15A discloses the amino acid sequence of the SW149.5 protein; Figure 15B discloses the amimo acid sequence of the CAB1.1 protein; Figure 15C discloses the nucleotide sequence of the CAB1 gene; Figure 15D discloses the amino acid sequence of the CAB1.2 protein; Figure 15E discloses the amino acid sequence of the CAB1.4CDRs; Figure 15F discloses the nucleotide sequence of the CAB1.4 CDRs; Figure 15G discloses the nucleotide sequence of the entire CAB1.4 gene, including BLA, etc; Figure 15H discloses the amino acid sequence of the CAB1.4 protein; Figure 15I discloses the nucleotide sequence of the CAB1.6 CDRs; Figure 15J discloses the nucleotide sequence of the entire CAB1.6 gene, including BLA, etc.; Figure 15K discloses the nucleotide sequence of the entire CAB1.6i gene, including BLA, etc.; Figure 15L discloses the nucleotide sequence of the CAB1.7 CDRs; Figure 15M discloses the nucleotide sequence of the entire CAB1.7 gene, including BLA, etc.; Figure 15N discloses the nucleotide sequence of the entire CAB1.7i gene, including BLA, etc; Figure 15O discloses the nucleotide sequence of the CAB1 CDRs; Figure 15P discloses the nucleotide sequence for the entire CAB1.2 gene, including BLA, etc; Figure 15Q discloses the amino acid sequence for the SW149.5 CDRs; Figure 15R discloses the nucleotide sequence for the SW149.5 CDRs; Figure 15S discloses the nucleotide sequence for the entire SW149.5, including BLA, etc.; Figure 15T discloses the nucleotide sequence for BLA; Figure 15U discloses the nucleotide sequence for CAB1.1.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are used as described below.

"CAB" molecule shall mean a targeted agent that binds to a CEA target or microtarget and has a modified sequence and whose unmodified sequence comprises the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2. SEQ ID NO:1 sets forth the amino acid sequence of the unmodified CDR portion of the CAB molecule of the present invention as shown in Figure 1 SEQ ID NO:2 sets forth a CAB molecule that includes BLA as shown in Figure 2 and position numbering shall be with respect to SEQ ID NO:1 and SEQ ID NO:2, as set forth in Figure 1 and Figure 2, respectively. CAB designations may be followed by a number to designate specific combinations of modifications of the present invention. For example, as set forth above, and throughout the rest of the application, CAB1.6 shall refer to a CAB molecule having the following modifications: T100L, S184D and S226D, wherein position numbering is with respect to SEQ ID NO:1; or a CAB molecule having the following mutations: K3Q, R13K, T16G, L37V, T100L, M146V, W181V, S184D and S226D, wherein position numbering is with respect to SEQ ID NO:2. Also, for example, CAB1.7i shall refer to a CAB molecule having the following modifications: K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D, S226D, K265A and S568A, wherein position numbering is with respect to SEQ ID NO:2 as shown in Figure 2.

A "targeted agent" is a chemical entity that binds selectively to a microtarget of interest. Examples of targeted agents are antibodies, peptides and inhibitors. Of interest are targeted enzymes that have a desired catalytic activity and that can bind to one or

more target structures with high affinity and selectivity. Targeted enzymes retain at least most of their activity while bound to a target.

A "binding moiety" is a part of a targeted agent (or an ADEPT costruct, e.g., CAB molecule) that binds a microtarget. A binding moiety can comprise more than one region, either contiguous or non-contiguous, of the CAB.

An "active moiety" is a part of a targeted agent (or an ADEPT construct, e.g., CAB molecule) that confers functionality to the agent. An active moiety can comprise more than one region, either contiguous or non-contiguous, of, for example, a CAB molecule. In particular, an active moiety can be a beta-lactamase.

The term "protein" is used interchangeably here with the terms "peptide" and "polypeptide," and refers to a molecule comprising two or more amino acid residues joined by a peptide bond.

The terms "cell", "cell line", and "cell culture" can be used interchangeably and all such designations include progeny. The words "transformants" or "transformed cells" include the primary transformed cell and cultures derived from that cell without regard to the number of transfers. All progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same functionality as screened for in the originally transformed cell are included in the definition of transformants. The cells can be prokaryotic or eukaryotic.

The term "oligonucleotide" as used herein is defined as a molecule comprised of two or more deoxyribonucleotides or ribonucleotides. The exact size will depend on many factors, which in turn depends on the ultimate function or use of the oligonucleotide. Oligonucleotides can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical synthesis by a method such as the phosphotriester method of Narang et al., 1979, Meth. Enzymol. 68:90-99; the phosphodiester method of Brown et al., 1979, Meth. Enzymol. 68:109-151; the diethylphosphoramidite method of Beaucage et al., 1981, Tetrahedron Lett. 22:1859-1862; and the solid support method of U.S. Pat. No. 4,458,066, each incorporated herein by reference. A review of synthesis methods is provided in

Goodchild, 1990, Bioconjugate Chemistry 1(3):165-187, incorporated herein by reference.

The term "primer" as used herein refers to an oligonucleotide capable of acting as a point of initiation of synthesis when placed under conditions in which primer extension is initiated. Synthesis of a primer extension product that is complementary to a nucleic acid strand is initiated in the presence of the requisite four different nucleoside triphosphates and a DNA polymerase in an appropriate buffer at a suitable temperature. A "buffer" includes a buffer, cofactors (such as divalent metal ions) and salt (to provide the appropriate ionic strength), adjusted to the desired pH.

A primer that hybridizes to the non-coding strand of a gene sequence (equivalently, is a subsequence of the noncoding strand) is referred to herein as an "upstream" or "forward" primer. A primer that hybridizes to the coding strand of a gene sequence is referred to herein as an "downstream" or "reverse" primer.

Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, cysteine, glycine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Standard three-letter or one-letter amino acid abbreviations are used herein. Equivalent substitutions may be included within the scope of the claims.

The peptides, polypeptides and proteins of the invention can comprise one or more non-classical amino acids. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid (4-Abu), 2-aminobutyric acid (2- Abu), 6-amino hexanoic acid (Ahx), 2-amino isobutyric acid (2-Aib), 3-amino propionoic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, ß-alanine, fluoro-amino acids, designer amino acids such as ß-methyl

amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general.

The term "Ab" or "antibody" refers to polyclonal and monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, immunoglobulins or antibody or functional fragments of an antibody that binds to a target antigen. Examples of such functional entities include complete antibody molecules, antibody fragments, such as Fv, single chain Fv, complementarity determining regions (CDRs), V_L (light chain variable region), V_H (heavy chain variable region) and any combination of those or any other functional portion of an immunoglobulin peptide capable of binding to target antigen.

The term "prodrug" refers to a compound that is converted via one or more enzymatically catalyzed steps into an active compound that has an increased pharmacological activity relative to the prodrug. A prodrug can comprise a pro-part or inactive moiety and a drug or active drug or detectable moiety. Optionally, the prodrug also contains a linker. For example, the prodrug can be cleaved by an enzyme to release an active drug. Alternatively, an enzyme could alter the prodrug to release a detectable moiety. In a more specific example, prodrug cleavage by the targeted enzyme releases the active drug into the vicinity of the target bound to the targeted enzyme. "Pro-part" and "inactive moiety" refer to the inactive portion of the prodrug after it has been converted. For example, if a prodrug comprises a PEG molecule linked by a peptide to an active drug, the pro-part is the PEG moiety with or without a portion of the peptide linker.

The term "drug" and "active drug" and "detectable moiety" refer to the active moieties of a prodrug. After cleavage of the prodrug by a targeted enzyme, the active drug acts therapeutically upon the targeted tumor, cell, infectious agent or other agent of disease. The detectable moiety acts as a diagnostic tool, and such detectable moieties are intended to be within the scope of the claims. The active drug can be any chemical entity that is able to kill a cell or inhibit cell proliferation.

The term "% sequence homology" is used interchangeably herein with the terms "% homology," "% sequence identity" and "% identity" and refers to the level of amino acid sequence identity between two or more peptide sequences, when aligned using a sequence alignment program. For example, as used herein, 80% homology means the same thing as 80% sequence identity determined by a defined algorithm, and accordingly, a homologue of a given sequence has greater than 80% sequence identity over a length of the given sequence. Exemplary levels of sequence identity include, but are not limited to, 60, 70, 80, 85, 90, 95, 98 or 99% or more sequence identity to a given sequence.

Exemplary computer programs that can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, *e.g.*, BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, which are well-known to one skilled and the art. *See also* Altschul *et al.*, 1990, *J. Mol. Biol.* 215: 403-10 and Altschul *et al.*, 1997, *Nucleic Acids Res.*, 25:3389-3402. Sequence searches are typically carried out using the BLASTP program when evaluating a given amino acid sequence relative to amino acid sequences in the GenBank Protein Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTP and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. *See* Altschul, *et al.*, 1997.

A preferred alignment of selected sequences in order to determine "% identity" between two or more sequences, is performed using for example, the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

In a first aspect, the invention is drawn to a CAB molecule comprising a modified amino acid sequence. In one embodiment, the CAB molecule has an amino acid sequence modified from the amino acid sequence set forth in SEQ ID NO:1, and the modification is at least one position selected from the group consisting of positions 100, 102, 104, 105, 107, 163, 165, 166, 184 and 226, wherein position numbering is with

respect to SEQ ID NO:1 as shown in Figure 1. In a preferred embodiment, the CAB molecule comprises modifications at positions 100, 184 and 226. In a preferred embodiment, the CAB molecule comprises modifications at positions 100, 102, 104, 105, 107, 163, 165, 166, 184 and 226.

In a preferred embodiment, the modification is at least one selected from the group consisting of T100L, T102L, P104A, Y105I, F107N, S163A, S165Y, Y166A, S184D and S226D, wherein position numbering is with respect to SEQ ID NO:1 as shown in Figure 1. In a preferred embodiment, the CAB molecule comprises a CAB1.6 molecule, the CAB1.6 molecule having the following modifications: T100L, S184D and S226D. In a preferred embodiment, the CAB molecule comprises a CAB1.7 molecule, the CAB1.7 molecule having the following modifications: T100L, T102L, P104A, Y105I, F107N, S163A, S165Y, Y166A, S184D and S226D.

In a preferred embodiment, the CAB molecule comprises the scFV portion of CAB1.6 (SEQ ID NO:5), or CAB1.7 (SEQ ID NO:6).

In a preferred embodiment, the CAB molecule further comprises a beta-lactamase molecule. In a preferred embodiment, the CAB molecule has an amino acid sequence modified from the amino acid sequence set forth in SEQ ID NO:2, and the modification is at least one position selected from the group consisting of positions: 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184, 226, 265 and 568, wherein position numbering is with respect to SEQ ID NO:2 as shown in Figure 2. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 146, 181, 184 and 226. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184 and 226. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184, 226, 268 and 568.

In a preferred embodiment, the CAB molecule further comprises a beta-lactamase molecule. In a preferred embodiment, the CAB molecule has an amino acid sequence modified from the amino acid sequence set forth in SEQ ID NO:2, and the modification is at least one position selected from the group consisting of positions: 3, 13, 16, 37, 100,

102, 104, 105, 107, 146, 163, 165, 166, 181, 184, 226, 265 and 568, wherein position numbering is with respect to SEQ ID NO:2 as shown in Figure 2. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 146, 181, 184 and 226. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184 and 226. In a preferred embodiment, the modifications are at positions 3, 13, 16, 37, 100, 102, 104, 105, 107, 146, 163, 165, 166, 181, 184, 226, 265 and 568.

In a preferred embodiment, the CAB molecule has modifications comprising at least one modification selected from the group consisting of K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D, S226D, K265A and S568A, wherein position numbering is with respect to SEQ ID NO:2 as shown in Figure 2. In a preferred embodiment, the CAB molecule comprises a CAB1.6 molecule, the CAB1.6 molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, M146V, W181V, S184D and S226D. In a preferred embodiment, the CAB molecule comprises a CAB1.6i molecule, the CAB1.6i molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, M146V, W181V, S184D, S226D, K265A and S568A. In a preferred embodiment, the CAB molecule comprises a CAB1.7 molecule, the CAB1.7 molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D and S226D. In a preferred embodiment, the CAB comprises a CAB1.7i molecule, the CAB1.7i molecule comprising the following modifications: K3Q, R13K, T16G, L37V, T100L, T102L, P104A, Y105I, F107N, M146V, S163A, S165Y, Y166A, W181V, S184D, S226D, K265A and S568A.

In a preferred embodiment, the CAB molecule comprises CAB1.6 (SEQ ID NO:7), CAB1.6i (SEQ ID NO:8), CAB1.7 (SEQ ID NO:9) or CAB1.7i (SEQ ID NO:10).

In another embodiment, the CAB is an MDTA as described in PCT Application Number US03/18200, filed June 12, 2002 and incorporated herein by reference in its entirety. Some of the CAB molecules of the present invention have been shown to preferentially bind to a microtarget present on a target relative to binding of a non-target.

The difference in binding can be caused by any difference between the target and non-target such as, for example, a difference in pH, oxygen pressure, concentration of solutes or analytes (*e.g.*, lactic acid, sugars or other organic or inorganic molecules), temperature, light or ionic strength. Preferential binding of the CABs of the current invention can be used to bind to a microtarget under a desired set of conditions, identify a target *in vitro*, *ex vivo*, *in situ* or *in vivo* (*e.g.*, a target tissue in a subject), kill a target cell or tissue, convert a prodrug into an active drug in or near a target tissue. It also can be used as surface catalysts, for example, a targeted laccase. Other uses include, *e.g.*, targeted generation of a compound (*e.g.*, H_2O_2 from glucose) and the targeted destruction of compounds (*e.g.*, a metabolite or signalling molecule from a particular tissue).

In one embodiment, the CAB is selected, made or modified using an affinity maturation method, *e.g.*, as described in PCT application, filed Juine 12, 2002 and incorporated herein by reference in its entirety.

In another embodiment, the CAB is selected, made or modified using a loop-grafting method, *e.g.*, as described in U.S. Pat. App. Ser. No. 10/170,387, filed June 12, 2002 and incorporated herein by reference in its entirety.

In another embodiment, the CAB is a multifunctional polypeptide, *e.g.*, as described in U.S. Pat. App. Ser. No. 10/170,729, filed June 12, 2002 and incorporated herein by reference in its entirety.

In another embodiment, the CABs of the invention are used for diagnostic or therapeutic application such as those disclosed, for example, in United States patent 4,975,278, which is incorporated herein by reference in its entirety, as well as methods well-known in the art.

In one embodiment, the CAB molecule further comprises an active moiety. The active moiety can be a molecule, or a part of a molecule, that has an activity. The activity can be any activity. Examples of types of activities that the active moiety can have include, for example, a detectable activity, an enzymatic activity, a therapeutic activity, a diagnostic activity, a toxic activity or a binding activity. The active moiety can be a discrete part of the CAB, for example, an enzyme that is fused or conjugated to the

binding moiety, or it can be an integral part of the CAB, for example, binding of the CAB to the microtarget can activate or inhibit an activity of the microtarget or the target, or the CAB can be a targeted enzyme of the type discussed below and in copending United States Patent Application Serial Numbers 10/022,073 and 10/022,097, incorporated herein by reference in their entireties.

In another embodiment, the active moiety exhibits enzymatic activity, *e.g.*, it is an enzyme or an active fragment or derivative of an enzyme. Of particular interest are enzymes that can be used to activate a prodrug in a therapeutic setting. A large number of enzymes with different catalytic modes of action have been used to activate prodrugs. *See, e.g.*, Melton & Knox Enzyme-prodrug strategies for cancer therapy (1999) and Bagshawe *et al., Curr Opin Immunol* 11:579 (1999). Examples of types of enzymes that can be used to make the CABs of the present invention include, but are not limited to, proteases, carboxypeptidases, β-lactamases, asparaginases, oxidases, hydrolases, lyases, lipases, cellulases, amylases, aldolases, phosphatases, kinases, tranferases, polymerases, nucleases, nucleotidases, laccases, reductases, and the like. *See, e.g.*, co-pending U.S. Pat. App. Ser. No. 09/954,385, filed September 12, 2001, incorporated herein by reference in its entirety. As such, CABs of the invention can, for example, exhibit protease, carboxypeptidase, β-lactamase, asparaginase, oxidase, hydrolase, lyase, lipase, cellulase, amylase, aldolase, phospatase, kinase, tranferase, polymerase, nuclease, nucleotidase, laccase or reductase activity or the like. Examples of enzymes that can be used are those that can activate a prodrug, discussed below, and those that can produce a toxic agent from a metabolite, *e.g.*, hydrogen peroxide from glucose. *See* Christofidou-Solomidou *et al*, 2000, *Am J Physiol Lung Cell Mol Physiol* 278:L794.

In one embodiment, the present invention provides a CAB further comprising a β-lactamase ("BLA"). In another embodiment, the BLA is a targeted enzyme as described in co-pending United States Patent Application Serial Numbers 10/022,073 and 10/022,097, incorporated herein by reference in their entirety.

BLA enzymes are widely distributed in both gram-negative and gram-positive bacteria. BLA sequences are well known. A representative example of a BLA sequence

is depicted in Figure 3. BLA enzymes vary in specificity, but have in common that they hydrolyze β-lactams, producing substituted β-amino acids. Thus, they confer resistance to antibiotics containing β-lactams. Because BLA enzymes are not endogenous to mammals, they are subject to minimal interference from inhibitors, enzyme substrates, or endogenous enzyme systems (unlike proteases), and therefore are particularly well-suited for therapeutic administration. BLA enzymes are further well-suited to the therapeutic methods of the present invention because of their small size (BLA from *E. cloacae* is a monomer of 39 kD; BLA from *E. coli* is a monomer of 30 kD) and because they have a high specific activity against their substrates and have optimal activity at 37° C. *See* Melton *et al.*, Enzyme-Prodrug Strategies for Cancer Therapy, Kluwer Academic/Plenum Publishers, New York (1999).

Examples of specific BLAs that can be used to make the CABs of the present invention include, but are not limited to, Class A, B, C or D β-lactamase, β-galactosidase, *see* Benito *et al.*, *FEMS Microbiol. Lett.* 123:107 (1994), fibronectin, glucose oxidase, glutathione S-transferase, *see* Napolitano *et al.*, *Chem. Biol.* 3:359 (1996) and tissue plasminogen activator, *see* Smith *et al.*, *J. Biol. Chem.* 270:30486 (1995). The β-lactamases have been divided into four classes based on their sequences. *See* Thomson *et al.*, 2000, *Microbes and Infection* 2:1225-35. The serine β-lactamases are subdivided into three classes: A (penicillinases), C (cephalosporinases) and D (oxacillnases). Class B β-lactamases are the zinc-containing or metallo β-lactamases. Any class of BLA can be utilized to generate an CAB of the invention.

In one embodiment of the invention, the BLA has a specific activity greater than about 0.01 U/pmol against nitrocefin using the assay described in United States Patent Application Serial Number 10/022,097. In another embodiment, the specific activity is greater than about 0.1 U/pmol. In another embodiment, the specific activity is greater than about 1 U/pmol. Preferably, these specific activities refer to the specific activity of the BLA when it is bound to a microtarget.

In one embodiment, the BLA enzyme in the CAB comprises the amino acid sequence set forth in SEQ ID NO:3. In another embodiment, the BLA enzyme in the

CAB is at least 50%, 60%, 70%, 80%, 90%, 95%, 98% or 99% or more identical to the sequence depicted in Figure 2.

In a preferred embodiment, the CAB is CAB1.6, CAB1.6i, CAB1.7 or CAB1.7i.

The targets bound by the CAB, or one or more binding moieties, can be any substance or composition to which a molecule can be made to bind to CEA. In one embodiment, the target is a surface. In one embodiment, the surface is a biological surface. In another embodiment, the biological surface is a surface of an organ. In another embodiment, the biological surface is a surface of a tissue. In another embodiment, the biological surface is a surface of a cell. In another embodiment, the biological surface is a surface of a diseased organ, tissue or cell. In another embodiment, the biological surface is a surface of a normal or healthy organ, tissue or cell. In another embodiment, the surface is a macromolecule in the interstitial space of a tissue. In another embodiment, the biological surface is the surface of a virus or pathogen. In another embodiment, the surface is a non-biological surface. In another embodiment, the non-biological surface is a surface of a medical device. In another embodiment, the medical device is a therapeutic device. In another embodiment, the therapeutic device is an implanted therapeutic device. In another embodiment, the medical device is a diagnostic device. In another embodiment, the diagnostic device is a well or tray.

Sources of cells or tissues include human, all other animals, bacteria, fungi, viruses and plant. Tissues are complex targets and refer to a single cell type, a collection of cell types or an aggregate of cells generally of a particular kind. Tissue may be intact or modified. General classes of tissue in humans include but are not limited to epithelial tissue, connective tissue, nerve tissue and muscle tissue.

In another embodiment, the target is a cancer-related target that expresses CEA or that has CEA bound to itself or that has CEA located in its vicinity. The cancer-related target can be any target that a composition of the invention binds to as part of the diagnosis, detection or treatment of a cancer or cancer-associated condition in a subject, for example, a cancerous cell, tissue or organ, a molecule associated with a cancerous cell, tissue or organ, or a molecule, cell, tissue or organ that is associated with a

cancerous cell, tissue or organ (*e.g.*, a tumor-bound diagnostic or therapeutic molecule administered to a subject or to a biopsy taken from a subject, or a healthy tissue, such as vasculature, that is associated with cancerous tissue).

In a second aspect, the invention is drawn to a nucleic acid encoding a CAB molecule as set forth herein. The nucleic acid can be, for example, a DNA or an RNA. The present invention also provides a plasmid comprising a nucleic acid encoding a polypeptide comprising all or part of a CAB. The plasmid can be, for example, an expression plasmid that allows expression of the polypeptide in a host cell or organism, or *in vitro*. The expression vector can allow expression of the polypeptide in, for example, a bacterial cell. The bacterial cell can be, for example, an *E. coli* cell.

Because of the redundancy in the genetic code, typically a large number of DNA sequences encode any given amino acid sequence and are, in this sense, equivalent. As described below, it may be desirable to select one or another equivalent DNA sequences for use in a expression vector, based on the preferred codon usage of the host cell into which the expression vector will be inserted. The present invention is intended to encompass all DNA sequences that encode the desired CAB.

An operable expression clone may be used and is constructed by placing the coding sequence in operable linkage with a suitable control sequence in an expression vector. The vector can be designed to replicate autonomously in the host cell or to integrate into the chromosomal DNA of the host cell. The resulting clone is used to transform a suitable host, and the transformed host is cultured under conditions suitable for expression of the coding sequence. The expressed CAB is then isolated from the medium or from the cells, although recovery and purification of the CAB may not be necessary in some instances.

Construction of suitable clones containing the coding sequence and a suitable control sequence employ standard ligation and restriction techniques that are well understood in the art. In general, isolated plasmids, DNA sequences or synthesized oligonucleotides are cleaved, modified and religated in the form desired. Suitable

restriction sites can, if not normally available, be added to the ends of the coding sequence so as to facilitate construction of an expression clone.

Site-specific DNA cleavage is performed by treating with a suitable restriction enzyme (or enzymes) under conditions that are generally understood in the art and specified by the manufacturers of commercially available restriction enzymes. *See, e.g.*, product catalogs from Amersham (Arlington Heights, IL), Roche Molecular Biochemicals (Indianapolis, IN), and New England Biolabs (Beverly, MA). Incubation times of about one to two hours at a temperature that is optimal for the particular enzyme are typical. After each incubation, protein is removed by extraction with phenol and chloroform; this extraction can be followed by ether extraction and recovery of the DNA from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. *See, e.g.*, Maxam *et al.*, 1980, Methods in Enzymology 65:499-560.

Ligations can be performed, for example, in 15-30 µl volumes under the following standard conditions and temperatures: 20 mM Tris-Cl, pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 33 µg/ml BSA, 10-50 mM NaCl, and either 40 µM ATP and 0.01-0.02 (Weiss) units T4 DNA ligase at 0° C (for ligation of fragments with complementary single-stranded ends) or 1mM ATP and 0.3-0.6 units T4 DNA ligase at 14°C (for "blunt end" ligation). Intermolecular ligations of fragments with complementary ends are usually performed at 33-100 µg/ml total DNA concentrations (5-100 nM total ends concentration). Intermolecular blunt end ligations (usually employing a 20-30 fold molar excess of linkers, optionally) are performed at 1 µM total ends concentration.

Correct ligations for plasmid construction can be confirmed using any suitable method known in the art. For example, correct ligations for plasmid construction can be confirmed by first transforming a suitable host, such as *E. coli* strain DG101 (ATCC 47043) or *E. coli* strain DG116 (ATCC 53606), with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or sensitivity or by using other markers, depending on the mode of plasmid construction, as

is understood in the art. Plasmids from the transformants are then prepared according to the method of Clewell *et al.*, 1969, Proc. Natl. Acad. Sci. USA 62:1159, optionally following chloramphenicol amplification. *See* Clewell, 1972, J. Bacteriol. 110:667. Alternatively, plasmid DNA can be prepared using the "Base-Acid" extraction method at page 11 of the Bethesda Research Laboratories publication Focus 5 (2), and very pure plasmid DNA can be obtained by replacing steps 12 through 17 of the protocol with CsCl/ethidium bromide ultracentrifugation of the DNA. As another alternative, a commercially available plasmid DNA isolation kit, *e.g.*, HISPEED™, QIAFILTER™ and QIAGEN® plasmid DNA isolation kits (Qiagen, Valencia CA) can be employed following the protocols supplied by the vendor. The isolated DNA can be analyzed by, for example, restriction enzyme digestion and/or sequenced by the dideoxy method of Sanger *et al.*, 1977, Proc. Natl. Acad. Sci. USA 74:5463, as further described by Messing *et al.*, 1981, Nuc. Acids Res. 9:309, or by the method of Maxam *et al.*, 1980, Methods in Enzymology 65:499.

The control sequences, expression vectors and transformation methods are dependent on the type of host cell used to express the gene. Generally, procaryotic, yeast, insect or mammalian cells are used as hosts. Procaryotic hosts are in general the most efficient and convenient for the production of recombinant proteins and are therefore preferred for the expression of the protein.

The procaryote most frequently used to express recombinant proteins is *E. coli*. However, microbial strains other than *E. coli* can also be used, such as bacilli, for example *Bacillus subtilis*, various species of *Pseudomonas* and *Salmonella*, and other bacterial strains. In such procaryotic systems, plasmid vectors that contain replication sites and control sequences derived from the host or a species compatible with the host are typically used.

For expression of constructions under control of most bacterial promoters, *E. coli* K12 strain MM294, obtained from the *E. coli* Genetic Stock Center under GCSC #6135, can be used as the host. For expression vectors with the $P_L N_{RBS}$ or P_L $T7_{RBS}$ control sequence, *E. coli* K12 strain MC1000 lambda lysogen, $N7N53cI857$ $SusP_{80}$, ATCC

39531, may be used. *E. coli* DG116 , which was deposited with the ATCC (ATCC 53606) on April 7, 1987, and *E. coli* KB2, which was deposited with the ATCC (ATCC 53075) on March 29, 1985, are also useful host cells. For M13 phage recombinants, *E. coli* strains susceptible to phage infection, such as *E. coli* K12 strain DG98 (ATCC 39768), are employed. The DG98 strain was deposited with the ATCC on July 13, 1984.

For example, *E. coli* is typically transformed using derivatives of pBR322, described by Bolivar *et al.*, 1977, Gene 2:95. Plasmid pBR322 contains genes for ampicillin and tetracycline resistance. These drug resistance markers can be either retained or destroyed in constructing the desired vector and so help to detect the presence of a desired recombinant. Commonly used procaryotic control sequences, i.e., a promoter for transcription initiation, optionally with an operator, along with a ribosome binding site sequence, include the ß-lactamase (penicillinase) and lactose (lac) promoter systems, *see* Chang *et al.*, 1977, Nature 198:1056, the tryptophan (trp) promoter system, *see* Goeddel *et al.*, 1980, Nuc. Acids Res. 8:4057, and the lambda-derived P_L promoter, *see* Shimatake *et al.*, 1981, Nature 292:128, and gene N ribosome binding site (N_{RBS}). A portable control system cassette is set forth in U.S. Patent No. 4,711,845, issued December 8, 1987. This cassette comprises a P_L promoter operably linked to the N_{RBS} in turn positioned upstream of a third DNA sequence having at least one restriction site that permits cleavage within six base pairs 3' of the N_{RBS} sequence. Also useful is the phosphatase A (phoA) system described by Chang *et al.*, in European Patent Publication No. 196,864, published October 8, 1986. However, any available promoter system compatible with procaryotes can be used to construct a expression vector of the invention.

In addition to bacteria, eucaryotic microbes, such as yeast, can also be used as recombinant host cells. Laboratory strains of *Saccharomyces cerevisiae*, Baker's yeast, are most often used, although a number of other strains are commonly available. While vectors employing the two micron origin of replication are common, *see* Broach, 1983, Meth. Enz. 101:307, other plasmid vectors suitable for yeast expression are known. *See, e.g.*, Stinchcomb *et al.*, 1979, Nature 282:39; Tschempe *et al.*, 1980, Gene 10:157; and

Clarke *et al.*, 1983, Meth. Enz. 101:300. Control sequences for yeast vectors include promoters for the synthesis of glycolytic enzymes. *See* Hess *et al.*, 1968, J. Adv. Enzyme Reg. 7:149; Holland *et al.*, 1978, Biotechnology 17:4900; and Holland *et al.*, 1981, J. Biol. Chem. 256:1385. Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase, *see* Hitzeman *et al.*, 1980, J. Biol. Chem. 255:2073, and those for other glycolytic enzymes, such as glyceraldehyde 3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase. Other promoters that have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism and enzymes responsible for maltose and galactose utilization.

Terminator sequences may also be used to enhance expression when placed at the 3' end of the coding sequence. Such terminators are found in the 3' untranslated region following the coding sequences in yeast-derived genes. Any vector containing a yeast-compatible promoter, origin of replication and other control sequences is suitable for use in constructing yeast expression vectors.

The coding sequence can also be expressed in eucaryotic host cell cultures derived from multicellular organisms. *See, e.g.*, Tissue Culture, Academic Press, Cruz and Patterson, editors (1973). Useful host cell lines include COS-7, COS-A2, CV-1, murine cells such as murine myelomas N51 and VERO, HeLa cells and Chinese hamster ovary (CHO) cells. Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as, for example, the commonly used early and late promoters from Simian Virus 40 (SV 40), *see* Fiers *et al.*, 1978, Nature 273:113, or other viral promoters such as those derived from polyoma, adenovirus 2, bovine papilloma virus (BPV) or avian sarcoma viruses, or immunoglobulin promoters and heat shock promoters.

Enhancer regions are also important in optimizing expression; these are, generally, sequences found upstream of the promoter region. Origins of replication may be obtained, if needed, from viral sources. However, integration into the chromosome is a common mechanism for DNA replication in eucaryotes.

Plant cells can also be used as hosts, and control sequences compatible with plant cells, such as the nopaline synthase promoter and polyadenylation signal sequences, *see* Depicker *et al.*, 1982, J. Mol. Appl. Gen. 1:561, are available. Expression systems employing insect cells utilizing the control systems provided by baculovirus vectors have also been described. *See* Miller *et al.*, in Genetic Engineering (1986), Setlow *et al.*, eds., Plenum Publishing, Vol. 8, pp. 277-97. Insect cell-based expression can be accomplished in *Spodoptera frugipeida*. These systems are also successful in producing recombinant enzymes.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described by Cohen, 1972, Proc. Natl. Acad. Sci. USA 69:2110, is used for procaryotes or other cells that contain substantial cell wall barriers. Infection with *Agrobacterium tumefaciens*, *see* Shaw *et al.*, 1983, Gene 23:315, is used for certain plant cells. For mammalian cells, the calcium phosphate precipitation method of Graham *et al.*, 1978, Virology 52:546 is preferred. Transformations into yeast are carried out according to the method of Van Solingen *et al.*, 1977, J. Bact. 130:946, and Hsiao *et al.*, 1979, Proc. Natl. Acad. Sci. USA 76:3829.

It may be desirable to modify the sequence of a DNA encoding a polypeptide comprising all or part of a CAB of the invention to provide, for example, a sequence more compatible with the codon usage of the host cell without modifying the amino acid sequence of the encoded protein. Such modifications to the initial 5-6 codons may improve expression efficiency. DNA sequences which have been modified to improve expression efficiency, but which encode the same amino acid sequence, are considered to be equivalent and encompassed by the present invention.

A variety of site-specific primer-directed mutagenesis methods are available and well-known in the art. *See, e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1989, second edition, chapter 15.51, "Oligonucleotide-mediated mutagenesis," which is incorporated herein by reference. The polymerase chain reaction (PCR) can be used to perform site-specific mutagenesis. In another technique now standard in the art, a synthetic oligonucleotide encoding the desired mutation is used as a primer to direct synthesis of a complementary nucleic acid sequence contained in a single-stranded vector, such as pBSM13+ derivatives, that serves as a template for construction of the extension product of the mutagenizing primer. The mutagenized DNA is transformed into a host bacterium, and cultures of the transformed bacteria are plated and identified. The identification of modified vectors may involve transfer of the DNA of selected transformants to a nitrocellulose filter or other membrane and the "lifts" hybridized with kinased synthetic mutagenic primer at a temperature that permits hybridization of an exact match to the modified sequence but prevents hybridization with the original unmutagenized strand. Transformants that contain DNA that hybridizes with the probe are then cultured (the sequence of the DNA is generally confirmed by sequence analysis) and serve as a reservoir of the modified DNA.

Once the polypeptide has been expressed in a recombinant host cell, purification of the polypeptide may be desired. A variety of purification procedures can be used.

In another embodiment, a nucleic acid encoding the CAB hybridizes to a nucleic acid complementary to a nucleic acid encoding any of the amino acid sequences disclosed herein under highly stringent conditions. The highly stringent conditions can be, for example, hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C and washing in 0.1xSSC/0.1 % SDS at 68° C (Ausubel *et al.*, eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Other highly stringent conditions can be found in, for example, *Current Protocols in Molecular Biology*, at pages 2.10.1-16 and *Molecular Cloning: A Laboratory Manual*, 2d ed., Sambrook *et al.* (eds.), Cold Spring Harbor Laboratory Press, 1989, pages 9.47-57. In

another embodiment, moderately stringent conditions are used. The moderately stringent conditions can be, for example, washing in 0.2xSSC/0.1% SDS at 42° C (Ausubel et al., 1989, *supra*). Other moderately stringent conditions can be found in, for example, *Current Protocols in Molecular Biology*, Vol. I, Ausubel *et al.* (eds.), Green Publishing Associates, Inc., and John Wiley & Sons, Inc., 1989, pages 2.10.1-16 and *Molecular Cloning: A Laboratory Manual*, 2d ed., Sambrook *et al.* (eds.), Cold Spring Harbor Laboratory Press, 1989, pages 9.47-57.

In a third aspect the present invention provides a method of treating a subject in need thereof comprising administering to a subject a CAB and a prodrug that is a substrate of the CAB. In another embodiment, the invention provides a method of treating a subject by administering to the subject a CAB, further comprising a BLA, and a prodrug that is converted by the BLA into an active drug. Examples of suitable prodrugs for this embodiment are provided in, *e.g.*, Melton *et al.*, Enzyme-Prodrug Strategies for Cancer Therapy, Kluwer Academic/Plenum Publishers, New York (1999), Bagshawe *et al.*, *Current Opinion in Immunology* 11:579-83 (1999) and Kerr *et al.*, *Bioconjugate Chem.* 9:255-59 (1998). In another embodiment, the CAB is specifically CAB1.6, CAB1.7 or CAB1.7i.

Examples of enzyme/prodrug/active drug combinations are found in, *e.g.*, Bagshawe *et al.*, *Current Opinions in Immunology*, 11:579-83 (1999); Wilman, "Prodrugs In Cancer Chemotherapy," *Biochemical Society Transactions*, 14, pp. 375-82 (615th Meeting, Belfast 1986) and V. J. Stella *et al.*, "Prodrugs: A Chemical Approach To Targeted Drug Delivery," *Directed Drug Delivery*, R. Borchardt *et al.* (ed), pp.247-67 (Humana Press 1985). In one embodiment, the prodrug is a peptide. Examples of peptides as prodrugs can be found in Trouet *et al.*, *Proc Natl Acad Sci USA* 79:626 (1982), and Umemoto *et al.*, *Int J Cancer* 43:677 (1989). These and other reports show that peptides are sufficiently stable in blood. Another advantage of peptide-derived prodrugs is their amino acid sequences can be chosen to confer suitable pharmacological properties like half-life, tissue distribution and low toxicity to the active drugs. Most

reports of peptide-derived prodrugs relied on relatively nonspecific activation of the prodrug by, for instance, lysosomal enzymes.

The prodrug can be one that is converted to an active drug in more than one step. For example, the prodrug can be converted to a precursor of an active drug by the CAB. The precursor can be converted into the active drug by, for example, the catalytic activity of one or more additional CABs, the catalytic activities of one or more other enzymes administered to the subject, the catalytic activity of one or more enzymes naturally present in the subject or at the target site in the subject (*e.g.*, a protease, a phosphatase, a kinase or a polymerase), by a drug that is administered to the subject or by a chemical process that is not enzymatically catalyzed (*e.g.*, oxidation, hydrolysis, isomerization or epimerization).

Most studies involving prodrugs are generated after programs with existing drugs are found to be problematic. In particular anticancer drugs were generally characterized by a very low therapeutic index. By converting these drugs into prodrugs with reduced toxicity and then selectively activating them in the diseased tissue, the therapeutic index of the drug was significantly increased. *See, e.g.*, Melton *et al.*, Enzyme-prodrug strategies for cancer therapy (1999), and Niculescu-Duvaz *et al., Anticancer Drug Des* 14:517 (1999).

The literature describes many methods to alter the substrate specificity of enzymes by protein engineering or directed evolution. Thus one skilled in the art is able to evolve the specificity of an enzyme to accommodate even structures that would be poor substrates for naturally-occurring enzymes. Accordingly, prodrugs can be designed even though the drugs were otherwise not amenable to a prodrug strategy.

A number of studies have been performed with toxins coupled to targeting agents (usually antibodies or antibody fragments). *See, e.g.,* Torchilin, *Eur J Pharm Sci* 11Suppl 2:S81 (2000) and Frankel *et al., Clin Cancer Res* 6:326 (2000). An alternative to the above is to convert these toxins into prodrugs and then selectively release them in the diseased tissue.

The prodrugs of this invention include, but are not limited to, aurstatins, camptothecins, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide - containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted by the enzyme of the conjugate into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, etoposide, temposide, adriamycin, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, cis-platinum and cis-platinum analogues, bleomycins, esperamicins (*see* U.S. Pat. No. 4,675,187), 5-fluorouracil, melphalan, other related nitrogen mustards and derivatives thereof. *See, e.g.*, U.S. Pat. No. 4,975,278.

In one embodiment of the invention, the CAB comprises an alkaline phosphatase (AP) that converts a 4'-phosphate derivative of the epipodophyl-lotoxin glucosides into an active anti-cancer drug. Such derivatives include etoposide-4'-phosphate, etoposide-4'-thiophosphate and teniposide-4'-phosphate. Other embodiments of the invention may include phosphate derivatives of these glucosides wherein the phosphate moiety is placed at other hydroxyl groups on the glucosides. According to another embodiment, however, the phosphate derivative used as a pro-drug in this invention is etoposide-4'-phosphate or etoposide-4'-thiophosphate. The targeted AP removes the phosphate group from the prodrug, releasing an active antitumor agent. The mitomycin phosphate prodrug of this embodiment may be an N^7-C_{1-8} alkyl phosphate derivative of mitomycin C or porfiromycin or pharmaceutically acceptable salts thereof. N^7 refers to the nitrogen atom attached to the 7-position of the mitosane nucleus of the parent drug. According to another embodiment, the derivative used is 7-(2'-aminoethylphosphate)mitomycin ("MOP"). Alternatively, the MOP compound may be termed, 9-methoxy-7-[[(phosphonooxy)ethyl]amino]mitosane disodium salt. Other embodiments of the invention may include the use pf N^7-alkyl mitomycin phosphorothioates as prodrugs.

In still another embodiment of the invention, the CAB comprises a penicillin amidase enzyme that converts a novel adriamycin prodrug into the active antitumor drug adriamycin. In another embodiment, the penicillin amidase is a penicillin V amidase ("PVA") isolated from *Fusarium oxysporum* that hydrolyzes phenoxyacetyl amide bonds. The prodrug utilized can be N-(p-hydroxyphenoxyacetyl)adriamycin ("APO"), which is hydrolyzed by the amidase to release the potent antitumor agent or adriamycin.

The present invention also comprises, for example, the use of the adriamycin prodrug, N-(p-hydroxyphenoxyacetyl)adriamycin and other related adriamycin prodrugs that can be derivatized in substantially the same manner. For example, use of the prodrug N-(phenoxyacetyl) adriamycin is also within the scope of the invention. In addition, it is to be understood that the adriamycin prodrugs of this invention include other N-hydroxyphenoxyacetyl derivatives of adriamycin, *e.g.*, substituted at different positions of the phenyl ring, as well as N-phenoxyacetyl derivatives containing substituents on the phenyl ring other than the hydroxyl group described herein.

Furthermore, the present embodiment encompasses the use of other amidases, such as penicillin G amidase, as part of the CAB as well as other prodrugs correspondingly derivatized such that the particular amidase can hydrolyze that prodrug to an active antitumor form. For example, when the CAB further comprises penicillin G amidase, the prodrug should contain a phenylacetylamide group (as opposed to the phenoxyacetylamide group of APO) because penicillin G amidases hydrolyze this type of amide bond (*see, e.g.*, A. L. Margolin *et al.*, *Biochim. Biophys Acta.* 616, pp. 283-89 (1980)). Thus, other prodrugs of the invention include N-(p-hydroxyphenylacetyl) adriamycin, N-(phenylacetyl) adriamycin and other optionally substituted N-phenylacetyl derivatives of adriamycin.

It should also be understood that the present invention includes any prodrug derived by reacting the amine group of the parent drug with the carboxyl group of phenoxyacetic acid, phenylacetic acid or other related acids. Thus, prodrugs of anthracyclines other than adriamycin that are capable of being derivatized and acting in substantially the same manner as the adriamycin prodrugs described herein falls within

the scope of this invention. For example, other prodrugs that can be produced and used in accordance with this invention include hydroxyphenoxyacetylamide derivatives, hydroxyphenylacetylamide derivatives, phenoxyacetylamide derivatives and phenylacetylamide derivatives of anthracyclines such as daunomycin and carminomycin. Other amine-containing drugs such as melphalan, mitomycin, aminopterin, bleomycin and dactinomycin can also be modified described herein to yield prodrugs of the invention.

Another embodiment of the invention involves a CAB form of the enzyme cytosine deaminase ("CD"). The deaminase enzyme catalyzes the conversion of 5-fluorocytosine ("5-FC"), a compound lacking in antineoplastic activity, to the potent antitumor drug, 5-fluorouracil ("5-FU").

Another embodiment of the method of this invention provides a method of combination chemotherapy using several prodrugs and a single CAB. According to this embodiment, a number of prodrugs are used that are all substrates for the same CAB. Thus, a particular CAB converts a number of prodrugs into cytotoxic form, resulting in increased antitumor activity at the tumor site.

There is often a requirement for extending the blood circulation half-lives of pharmaceutical peptides, proteins, or small molecules. Typically short half-lives—lasting minutes to hours—require not only frequent, but also high doses for therapeutic effect—often so high that initial peak doses cause side effects. Extending the half-life of such therapeutics permits lower, less frequent, and therefore potentially safer doses, which are cheaper to produce. Previously researchers have increased protein half-life by fusing them covalently to PEG, *see* U.S. Patent 5,711,944, human blood serum albumin, *see* U.S. Patent 5,766,883, or Fc fragments, *see* WO 00/24782. In addition, nonspecific targeting of drugs to human serum albumin has been accomplished by chemical coupling drugs *in vivo*. *See* U.S. Patent 5,843,440. Furthermore, in the case of cancer drugs it has been proposed that high molecular weight drugs may localize in tumors due to enhanced permeability and retention. Therefore, improvement in the therapeutic index of a drug can be obtained by linking the drug to a protein or other high molecular weight polymer.

In another embodiment the present invention provides a method of treating a condition in subject comprising administering to the subject a CAB with β-lactamase activity and a prodrug. In another embodiment, the CAB is targeted to a CEA expressing cell, tissue, tumor or organ. In another embodiment, the prodrug is converted by the CAB into an active drug. In another embodiment, the active drug is an alkylating agent. In another embodiment, the prodrug is an anticancer nitrogen mustard prodrug. In another embodiment, the active drug is melphalan. In another embodiment, the prodrug is C-Mel. *See* Kerr *et al.*, *Bioconjugate Chem.* 9:255-59 (1998). In another embodiment, the prodrug is vinca-cephalosporin or doxorubicin cephalosporin. *See* Bagshawe *et al.*, *Current Opinion in Immunology*, 11:579-83 (1999). Other prodrug/enzyme combinations that can be used in the present invention include, but are not limited to, those found in U.S. Patent No. 4,975,278 and Melton *et al.*, Enzyme-Prodrug Strategies for Cancer Therapy Kluwer Academic/Plenum Publishers, New York (1999).

In a fourth aspect, the invention is drawn to a pharmaceutical composition comprising a CAB molecule. The CABs, nucleic acids encoding them and, in certain embodiments, prodrugs described herein can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the active compound and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a CAB, prodrug or nucleic acid of interest. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of an active compound of interest. Such compositions

can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of a CAB, prodrug or nucleic acid of interest and one or more additional active compounds.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like) and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance

of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a

sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form

as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Typically, the amount of CAB to be delivered to a subject will depend on a number of factors, including, for example, the route of administration, the activity of the CAB, the degree to which it is specifically targeted to the desired cells, tissues or organs of the subject, the length of time required to clear the non-specifically bound CAB from the subject, the desired therapeutic effect, the body mass of the subject, the age of the subject, the general health of the subject, the sex of the subject, the diet of the subject, the subject's immune response to the CAB, other medications or treatments being administered to the subject, the severity of the disease and the previous or future anticipated course of treatment.

For applications in which a prodrug also is administered, other factors affecting the determination of a therapeutically effective dose will include, for example, the amount of prodrug administered, the activity of the prodrug and its corresponding active drug and the side effects or toxicities of the prodrug and the active drug.

Examples of ranges of mass of CAB/mass of subject include, for example, from about 0.001 to 30 mg/kg body weight, from about 0.01 to 25 mg/kg body weight, from about 0.1 to 20 mg/kg body weight, and from about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

In a particular example, a subject is treated with a CAB in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, preferably between about 3 to 7 weeks and preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of CAB may increase or decrease over the course of a particular treatment, and that the treatment

will continue, with or without modification, until a desired result is achieved or until the treatment is discontinued for another reason. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

In an embodiment of the present invention, a prodrug also is administered to the subject. It is understood that appropriate doses of prodrugs depend upon a number of factors within the ken of the ordinarily skilled physician, veterinarian or researcher. The dose(s) of the prodrug will depend, for example, on the same factors provided above as factors affecting the effective dose of the CAB. Exemplary doses include milligram or microgram amounts of the prodrug per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is furthermore understood that appropriate doses of a prodrug depend upon the potency of the prodrug with respect to the desired therapeutic effect. When one or more of these prodrugs is to be administered to an animal (*e.g.*, a human), a physician, veterinarian or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained.

Preferably, the CAB is administered to the subject, then the prodrug is administered. More preferably, the time between the administration of the CAB and administration of the prodrug is sufficient to allow the CAB to accumulate at its target site by binding to its target, and to allow unbound CAB to be cleared from the non-targeted portions of the subject's body. Most preferably, the ratio of target-bound CAB to unbound CAB in the subject's body will be at or near its maximum when the prodrug is administered. The time necessary after administration of the CAB to reach this point is called the clearing time. The clearing time can be determined or approximated in an experimental system by, for example, administering a detectable CAB (*e.g.*, a radiolabeled or fluorescently labeled CAB) to a subject and simultaneously measuring the amount of enzyme at the target site and at a non-targeted control site at timed intervals. For some prodrugs, particularly those whose counterpart active drugs are highly toxic, it

may be more important to ensure that the levels of unbound CAB in the subject's system are below a certain threshold. This too can be determined experimentally, as described above.

In one embodiment, administration of the prodrug is systemic. In another embodiment, administration of the prodrug is at or near the target to be bound.

The pharmaceutical compositions can be included in a container, pack, dispenser or kit together with instructions for administration.

EXAMPLES

Example 1: Stabilization of an scFv

Construction of pME27.1

Plasmid pME27.1 was generated by inserting a Bgl I-EcoRV fragment encoding a part of the pelB leader, the CAB1-scFv and a small part of BLA into the expression vector pME25 (see, Figure 6). The insert, encoding for the CAB1-scFv has been synthesized by Aptagen (Herndon, VA) based on the sequence of the scFv MFE-23 that was described in [Boehm, M. K., A. L. Corper, T. Wan, M. K. Sohi, B. J. Sutton, J. D. Thornton, P. A. Keep, K. A. Chester, R. H. Begent and S. J. Perkins (2000) *Biochem J* 346 Pt 2, 519-28, Crystal structure of the anti-(carcinoembryonic antigen) single-chain Fv antibody MFE-23 and a model for antigen binding based on intermolecular contacts]. Both the plasmid containing the synthetic gene (pPCR-GME1) and pME25 were digested with BglI and EcoRV, gel purified and ligated together with Takara ligase. Ligation was transformed into TOP10 (Invitrogen, Carlsbad, CA) electrocompetent cells, plated on LA medium containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime.

Plasmid pME27.1 contains the following features:

P lac:	4992-5113 bp
pel B leader:	13-78
CAB 1 scFv:	79-810
BLA:	811-1896

T7 term.:	2076-2122
CAT:	3253-3912

A schematic of plasmid pME27.1 can be found in Figure 6A. The CAB1 sequence, indicating heavy and light chain domains, can be found in Figure 6B; the amino acid sequence can also be found in Figure 6D, with linker and BLA.

Choosing mutations for mutagenesis

The sequence of the vH and vL sequences of CAB1-scFv were compared with a published frequency analysis of human antibodies (Boris Steipe (1998) Sequenzdatenanalyse. *("Sequence Data Analysis", available in German only)* in *Bioanalytik* eds. H. Zorbas und F. Lottspeich, Spektrum Akademischer Verlag. S. 233-241). The authors aligned sequences of variable segments of human antibodies as found in the Kabat data base and calculated the frequency of occurrence of each amino acid for each position. These alignments can be seen in Figure 8. Specifically, Figure 8A shows an alignment of the observed frequencies of the five most abundant amino acids in alignment of human sequences in the heavy chain. Figure 8B shows an alignment of the observed frequencies of the five most abundant amino acids in alignment of human sequences in the light chain.

We compared these frequencies with the actual amino acid sequence of CAB1 and identified 33 positions that fulfilled the following criteria:

- The position is not part of a CDR as defined by the Kabat nomenclature.
- The amino acid found in CAB1-scFv is observed in the homologous position in less than 10% of human antibodies
- The position is not one of the last 6 amino acids in the light chain of scFv.

The resulting 33 positions were chosen for combinatorial mutagenesis.

Mutagenic oligonucleotides were synthesized for each of the 33 positions such that the targeted position would be changed from the amino acid in CAB1-scFv to the most abundant amino acid in the homologous position of a human antibody. Figure 6B

shows the sequence of CAB1-scFv, the CDRs and the mutations that were chosen for combinatorial mutagenesis.

Construction of library NA05

Table 1 listing the sequences of 33 mutagenic oligonucleotides that were used to generate combinatorial library NA05:

Table 1:

pos. (pME27) MFE-23 (VH)		count residues to be changed	QuikChange multi primer
3K	Q	nsa147.1fp	CGGCCATGGCCCAGGTGCAGCTGCAGCAGTCTGGGGC
13R	K	nsa147.2fp	CTGGGGCAGAACTTGTGAAATCAGGGACCTCAGTCAA
14S	P	nsa147.3fp	GGGCAGAACTTGTGAGGCCGGGGACCTCAGTCAAGTT
16T	G	nsa147.4fp	AACTTGTGAGGTCAGGGGGCTCAGTCAAGTTGTCCTG
28N	T	nsa147.5fp	GCACAGCTTCTGGCTTCACCATTAAAGACTCCTATAT
29I	F	nsa147.6fp	CAGCTTCTGGCTTCAACTTTAAAGACTCCTATATGCA
30K	S	nsa147.7fp	CTTCTGGCTTCAACATTAGCGACTCCTATATGCACTG
37L	V	nsa147.8fp	ACTCCTATATGCACTGGGTGAGGCAGGGGCCTGAACA
40G	A	nsa147.9fp	TGCACTGGTTGAGGCAGGCGCCTGAACAGGGCCTGGA
42E	G	nsa147.10fp	GGTTGAGGCAGGGGCCTGGCCAGGGCCTGGAGTGGAT
67K	R	nsa147.11fp	CCCCGAAGTTCCAGGGCCGTGCCACTTTTACTACAGA
68A	F	nsa147.12fp	CGAAGTTCCAGGGCAAGTTCACTTTTACTACAGACAC
70F	I	nsa147.13fp	TCCAGGGCAAGGCCACTATTACTACAGACACATCCTC
72T	R	nsa147.14fp	GCAAGGCCACTTTTACTCGCGACACATCCTCCAACAC
76S	K	nsa147.15fp	TTACTACAGACACATCCAAAAACACAGCCTACCTGCA
97N	A	nsa147.16fp	CTGCCGTCTATTATTGTGCGGAGGGGACTCCGACTGG
98E	R	nsa147.17fp	CCGTCTATTATTGTAATCGCGGGACTCCGACTGGGCC

136	E	Q	nsa147.18fp CTGGCGGTGGCGGATCACAGAATGTGCTCACCCAGTC
137	N	S	nsa147.19fp GCGGTGGCGGATCAGAAAGCGTGCTCACCCAGTCTCC
142	S	P	nsa147.20fp GAAAATGTGCTCACCCAGCCGCCAGCAATCATGTCTGC
144	A	S	nsa147.21fp TGCTCACCCAGTCTCCAAGCATCATGTCTGCATCTCC
146	M	V	nsa147.22fp CCCAGTCTCCAGCAATCGTGTCTGCATCTCCAGGGGA
152	E	Q	nsa147.23fp TGTCTGCATCTCCAGGGCAGAAGGTCACCATAACCTG
153	K	T	nsa147.24fp CTGCATCTCCAGGGGAGACCGTCACCATAACCTGCAG
170	F	Y	nsa147.25fp TAAGTTACATGCACTGGTACCAGCAGAAGCCAGGCAC
181	W	V	nsa147.26fp GCACTTCTCCCAAACTCGTGATTTATAGCACATCCAA
194	A	D	nsa147.27fp TGGCTTCTGGAGTCCCTGATCGCTTCAGTGGCAGTGG
200	G	K	nsa147.28fp CTCGCTTCAGTGGCAGTAAATCTGGGACCTCTTACTC
205	Y	A	nsa147.29fp GTGGATCTGGGACCTCTGCGTCTCTCACAATCAGCCG
212	M	L	nsa147.30fp CTCTCACAATCAGCCGACTGGAGGCTGAAGATGCTGC
217	A	E	nsa147.31fp GAATGGAGGCTGAAGATGAAGCCACTTATTACTGCCA
219	T	D	nsa147.32fp AGGCTGAAGATGCTGCCGATTATTACTGCCAGCAAAG
234	A	G	nsa147.33fp ACCCACTCACGTTCGGTGGCGGCACCAAGCTGGAGCT

The QuikChange multi site-directed mutagenesis kit (QCMS; Stratagene Catalog # 200514) was used to construct the combinatorial library NA05 using 33 mutagenic primers. The primers were designed so that they had 17 bases flanking each side of the codon of interest based on the template plasmid pME27.1. The codon of interest was changed to encode the appropriate consensus amino acid using an *E.coli* codon usage table. All primers were designed to anneal to the same strand of the template DNA (i.e., all were forward primers in this case). The QCMS reaction was carried out as described in the QCMS manual with the exception of the primer concentration used; the QCMC manual recommends using 50ng of each primer in the reaction, whereas we used 3 ng of each primer. Other primer amounts may be used. In particular, the reaction contained 50-100 ng template plasmid (pME27.1; 5178bp), 1 µl of primer mix (10 µM stock of all primers combined containing 0.3 µM each primer), 1 µl dNTPs (QCMS kit), 2.5 µl 10x

QCMS reaction buffer, 18.5 µl deoinized water and 1 µl enzyme blend (QCMS kit) for a total volume of 25 µl. The thermocycling program was 1 cycle at 95°C for 1 min., followed by 30 cycles of 95°C for 1 min., 55°C for 1 min. and then 65°C for 10 minutes. *Dpn*I digestion was performed by adding 1 µl *Dpn*I (provided in the QCMS kit), incubation at 37°C for 2 hours, addition of another 1 µl *Dpn*I, and incubation at 37°C for an additional 2 hours. 1 µl of the reaction was transformed into 50 µl of TOP10 electrocompetent cells from Invitrogen. 250 µl of SOC was added after electroporation, followed by a 1 hr incubation with shaking at 37°C. Thereafter, 10-50 µl of the tranformation mix was plated on LA plates with 5ppm chloramphenicol (CMP) or LA plates with 5ppm CMP and 0.1ppm of cefotaxime (CTX) for selection of active BLA clones. The active BLA clones from the CMP + CTX plates were used for screening whereas the random library clones from the CMP plates were sequenced to assess the quality of the library.

16 randomly chosen clones were sequenced. The clones contained different combinations of 1 to 7 mutations.

Screen for improved expression

When TOP10/pME27.1 is cultured in LB medium at 37 C then the concentration of intact fusion protein peaks after one day and most of the fusion protein is degraded by host proteases after 3 days of culture. Degradation seems to occur mainly in the scFv portion of the CAB1 fusion protein as the cultures contain significant amounts of free BLA after 3 days, which can be detected by Western blotting, or a nitrocefin (Oxoid, New York) activity assay. Thus we applied a screen to library NA05 that was able to detect variants of CAB1-scFv that would resist degradation by host proteases over 3 days of culture at 37 C.

Library NA05 was plated onto agar plates with LA medium containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime (Sigma). 910 colonies were transferred into a total of 10 96-well plates containing 100 ul/well of LA medium containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime. Four wells in each plate were inoculated with

TOP10/pME27.1 as control and one well per plate was left as a blank. The plates were grown overnight at 37 C. The next day the cultures were used to inoculate fresh plates (production plates) containing 100 ul of the same medium using a transfer stamping tool and glycerol was added to the master plates which were stored at –70 C. The production plates were incubated in a humidified shaker at 37C for 3 days. 100 ul of BPER (Pierce, Rockford, IL) per well was added to the production plate to release protein from the cells. The production plate was diluted 100-fold in PBST (PBS containing 0.125% Tween-20) and BLA activity was measured by transferring 20 ul diluted lysate into 180 ul of nitrocephin assay buffer (0.1 mg/ml nitrocephin in 50 mM PBS buffer containing 0.125% octylglucopyranoside (Sigma)) and the BLA activity was determined at 490 nm using a Spectramax plus plate reader (Molecular Devices, Sunnyvale, CA).

Binding to CEA (carcinoembryonic antigen, Biodesign Intl., Saco, Maine) was measured using the following procedure: 96-well plates were coated with 100 ul per well of 5 ug/ml of CEA in 50 mM carbonate buffer pH 9.6 overnight. The plates were washed with PBST and blocked for 1-2 hours with 300 ul of casein (Pierce, Rockford, IL). 100 ul of sample from the production plate diluted 100-1000 fold was added to the CEA-coated plate and the plates were incubated for 2 h at room temperature. Subsequently, the plates were washed four times with PBST and 200 ul nitrocefin assay buffer was added, and the BLA activity was measured as described above.

The BLA activity that was determined by the CEA-binding assay and the total BLA activity found in the lysate plates were compared and variants were identified that showed high levels of total BLA activity and high levels of CEA-binding activities.

The winners were confirmed in 4 replicates using a similar protocol: the winners were cultured in 2 ml of LB containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime for 3 days. Protein was released from the cells using BPER reagent. The binding assay was performed as described above but different dilutions of culture lysate were tested for each variant. Fig. 7A shows binding curves. Culture supernatants were also analyzed by SDS polyacrylamide electrophoresis. Fig. 7B shows the electropherogram of 7 variants from NA05. The band of the fusion protein is labeled for variant NA05.6. Table 2 shows

a ranking of 6 variants. The data were normalized and a performance index was calculated. The data clearly show that NA05.6 produces significantly larger quantities of fusion protein compared to the fusion construct pME27.1.

Table 2 showing the sequence of 6 variants with the largest improvement in stability:

Clone	mutations
NA05.6	R13K, T16G, W181V
NA05.8	R13K, F170Y, A234G
NA05.9	K3Q, S14P, L37V, E42G, E136Q, M146V, W181V, A234G
NA05.10	K3Q, L37V, P170Y, W181V
NA05.12	K3Q, S14P, L37V, M146V
NA05.15	M146V, F170Y, A194D

Construction of library NA06

Clone NA05.6 was chosen as the best variant and was used as the template for a second round of combinatorial mutagenesis; clone NA05.6 was designated CAB1.1. A subset of the same mutagenic primers that had been used to generate library NA05 to generate combinatorial variants with the following mutations: K3Q, L37V, E42G, E136Q, M146V, F170Y, A194D, A234G, was used; the mutations had been identified in other winners from library NA05. The primer encoding mutation S14P was not used as its sequence overlapped with mutations R13K and T16G present in NA05.6 (CAB1.1). A combinatorial library was constructed using QuikChange Multisite as described above and was called NA06. The template was pNA05.6 and 1 µl of primers mix (10 µM stock of all primers combined containing 1.25 µM each primer) were used.

Screening of library NA06

The screen was performed as described above with the following modifications:

291 variants were screened on 3 96-well plates. 10 µl sample from the lysate plates was added to 180 µl of 10 µg/ml thermolysin (Sigma) in 50 mM imidazole buffer pH 7.0 containing 0.005% Tween-20 and 10 mM calcium chloride. This mixture was incubated for 1 h at 37C to hydrolyze unstable variants of NA05.6 (CAB1.1). This protease-treated sample was used to perform the CEA-binding assay as described above.

Promising variants were cultured in 2 ml medium as described above and binding curves were obtained for samples after thermolysin treatments. Figure 7C shows binding curves for selected clones. A number of variants retain much more binding activity after thermolysin incubation than the parent NA05.6 (CAB1.1)

Table 3 shows 6 variants significantly more protease resistant than NA05.6 (CAB1.1):

Clone	Mutations
NA06.2	R13K, T16G, W181V, L37V, E42G, A194D
NA06.4	R13K, T16G, W181V, L37V, M146V
NA06.6	R13K, T16G, W181V, L37V, M146V, K3Q
NA06.10	R13K, T16G, W181V, L37V, M146V, A194D
NA06.11	R13K, T16G, W181V, L37V, K3Q, A194D
NA06.12	R13K, T16G, W181V, L37V, E136Q

All 6 variants have the mutation L37V; the mutation was rare in randomly chosen clones from the same library. Further testing showed that variant NA06.6 had the highest level of total BLA activity and the highest protease resistance of all variants. NA06.6 was chosen and designated CAB1.2.

Example 2: Generation of an scFV that has pH-dependent binding

Choosing positions for mutagenesis

The 3D structure of the scFv portion of NA06.6 (CAB1.2) was modeled based on the published crystal structure of a close homologue, MFE-23 [Boehm, M. K., A. L. Corper, T. Wan, M. K. Sohi, B. J. Sutton, J. D. Thornton, P. A. Keep, K. A. Chester, R. H. Begent and S. J. Perkins (2000) *Biochem J* 346 Pt 2, 519-28, Crystal structure of the anti-(carcinoembryonic antigen) single-chain Fv antibody MFE-23 and a model for antigen binding based on intermolecular contacts] using the software package MOE (Chemical Computing Group, Montreal, Canada) and using default parameters. A space-filling model of the structure was visually inspected. Side chains in the CDRs were ranked as follows: 0 = buried, 1= partially exposed and 2=completely exposed. Side chain distance to CDR3 was ranked as follows: 0= side chain is in CDR3, 1= side chain is one amino acid away from CDR3 and 2=side chain is two amino acids away from CDR3. In a few cases, residues flanking the CDRs were included if they fit the distance and exposure criteria.

Based on this ranking, the following side chains were targeted for mutagenesis:

a) exposure = 2 and distance = 2 or smaller

b) exposure = 1 and distance <2

40 positions in the CDRs matched these criteria.

Fig. 10 shows the CDRs and the residues that were chosen for mutagenesis.

Table 4 shows the criteria and position of the 40 sites that were chosen for mutagenesis.

Construction of library NA08

A combinatorial library was constructed where the 40 selected positions were randomly replaced with aspartate or histidine. The substitutions were chosen as it has been reported that ionic interactions between histidine side chains and carboxyl groups form the structural basis for the pH-dependence of the interaction between IgG molecules

and the Fc receptor [Vaughn, D. E. and P. J. Bjorkman (1998) *Structure* 6, 63-73., Structural basis of pH-dependent antibody binding by the neonatal Fc receptor].

The QuikChange multi site directed mutagenesis kit (QCMS; Stratagene Catalog # 200514) was used to construct the combinatorial library NA08 using 40 mutagenic primers. The primers were designed so that they had 17 bases flanking each side of the codon of interest based on the template plasmid NA06.6 (CAB1.2). The codon of interest was changed to the degenerate codon SAT to encode for aspartate and histidine. All primers were designed to anneal to the same strand of the template DNA (i.e., all were forward primers in this case). The QCMS reaction was carried out as described in the QCMS manual with the exception of the primer concentration used; the manual recommends using 50-100ng of each primer in the reaction, whereas significantly lower amounts of each primer were used in this library as this results in a lower parent template background. In particular, 0.4μM of all primers together were used. The individual degenerate primer concentration in the final reaction was 0.01 μM (approximately 2.5ng).

The QCMS reaction contained 50-100 ng template plasmid (NA06.6, 5178bp), 1 μl of primer mix (10μM stock of all primers to give the desired primer concentration mentioned above), 1 μl dNTPs (QCMS kit), 2.5 μl 10x QCMS reaction buffer, 18.5 μl deoinized water, and 1 μl enzyme blend (QCMS kit), for a total volume of 25 μl. The thermocycling program was 1 cycle at 95°C for 1 min., followed by 30 cycles of 95°C for 1 min., 55°C for 1 min. and 65°C for 10 minutes. *Dpn*I digestion was performed by adding 1 μl *Dpn*I (provided in the QCMS kit), incubating at 37°C for 2 hours, adding of 0.5 μl *Dpn*I and then incubating at 37°C for an additional 2 hours. 1 μl of each reaction was transformed into 50 μl of TOP10 electrocompetent cells from Invitrogen. 250 μl of SOC was added after electroporation, followed by a 1 hr incubation with shaking at 37°C. Thereafter, 10-50 μl of the transformation mix was plated on LA plates with 5ppm chloramphenicol (CMP) or LA plates with 5ppm CMP and 0.1ppm of cefotaxime (CTX) for selection of active BLA clones. The number of colonies obtained on both types of plates was comparable (652 on the CMP plate and 596 colonies on the CMP + CTX plate for 10 μl of the transformation mix plated). Active BLA clones from the CMP + CTX

plates were used for screening, whereas random library clones from the CMP plates were sequenced to assess the quality of the library.

Primers for the reaction are shown in Table 4:

Table 4 Primers for CDRs:

Residue	CDRs	position	exposure	distance to CDR3	primer sequence
K		30	2	2	cttctggcttcaacattsatgactcctatatgcactg
D	H1	31	2	1	ctggcttcaacattaaasattcctatatgcactgggt
S	H1	32	1	1	gcttcaacattaaagacsattatatgcactgggtgag
Y	H1	33	2	1	tcaacattaaagactccsatatgcactgggtgaggca
H	H1	35	1	1	ttaaagactcctatatgsattgggtgaggcaggggcc
W	H2	50	2	1	gcctggagtggattggasatattgatcctgagaatgg
D	H2	52	2	2	agtggattggatggattsatcctgagaatggtgatac
E	H2	54	2	2	ttggatggattgatcctsataatggtgatactgaata
N	H2	55	2	2	gatggattgatcctgagsatggtgatactgaatatgc
D	H2	57	2	1	ttgatcctgagaatggtsatactgaatatgccccgaa
T	H2	58	1	1	atcctgagaatggtgatsatgaatatgccccgaagtt
E	H2	59	2	1	ctgagaatggtgatactsattatgccccgaagttcca
P	H2	62	2	1	gtgatactgaatatgccsataagttccagggcaaggc
K	H2	63	2	3	atactgaatatgccccgsatttccagggcaaggccac
Q	H2	65	2	2	aatatgccccgaagttcsatggcaaggccacttttac
E		98	1	0	ccgtctattattgtaatsatgggactccgactgggcc
G		99	1	0	tctattattgtaatgagsatactccgactgggccgta
T	H3	100	2	0	attattgtaatgaggggsatccgactgggccgtacta
P	H3	101	2	0	attgtaatgaggggactsatactgggccgtactactt
T	H3	102	2	0	gtaatgaggggactccgsatgggccgtactactttga
G	H3	103	2	0	atgaggggactccgactsatccgtactactttgacta

Residue	CDRs	position	exposure	distance to CDR3	primer sequence
P	H3	104	2	0	aggggactccgactgggsattactactttgactactg
Y	H3	106	2	0	ctccgactgggccgtacsattttgactactggggcca
S	L1	162	2	2	taacctgcagtgccagcsatagtgtaagttacatgca
S	L1	163	2	1	cctgcagtgccagctcasatgtaagttacatgcactg
V	L1	164	1	1	gcagtgccagctcaagtsatagttacatgcactggtt
S	L1	165	2	1	gtgccagctcaagtgtasattacatgcactggttcca
Y	L1	166	2	1	ccagctcaagtgtaagtsatatgcactggttccagca
Y		183	1	0	ctcccaaactcgtgattsatagcacatccaacctggc
S	L2	184	2	0	ccaaactcgtgatttatsatacatccaacctggcttc
T	L2	185	1	1	aactcgtgatttatagcsattccaacctggcttctgg
S	L2	186	2	2	tcgtgatttatagcacasataacctggcttctggagt
N	L2	187	2	1	tgatttatagcacatccsatctggcttctggagtccc
A	L2	189	1	1	atagcacatccaacctgsattctggagtccctgctcg
S	L2	190	2	1	gcacatccaacctggctsatggagtccctgctcgctt
R	L3	225	2	2	cttattactgccagcaasattctagttacccactcac
S	L3	226	2	2	attactgccagcaaagasatagttacccactcacgt
S	L3	227	1	2	actgccagcaaagatctsattacccactcacgttcg
Y	L3	228	1	2	gccagcaaagatctagtsatccactcacgttcggtg
L	L3	230	1	2	aaagatctagttacccasatacgttcggtgctggcac

Sequencing of variants

Variants were grown overnight with shaking at 37°C in 5mL cultures of LA containing 5ppm of CMP. Miniprep DNA was prepared using a Qiagen kit and the BLA gene within each clone was sequenced using the M13 reverse and nsa154f primers.

M13 reverse: CAGGAAACAGCTATGAC

nsa154f: GGACCACGGTCACCGTCTCCTC

Screen pH-dependent binding

Library NA08 was plated onto agar plates with LA medium containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime (Sigma). 552 colonies were transferred into a total of six 96-well plates containing 100 ul/well of LA medium containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime. Four wells in each plate were inoculated with TOP10/NA06.6 as a reference. The plates were grown overnight at 37 C. The next day the cultures were used to inoculate fresh plates (production plates) containing 100 ul of the same medium using a transfer stamping tool and glycerol was added to the master plates which were stored at –70 C. The production plates were incubated in a humidified shaker at 37C for 2 days. 100 ul of BPER (Pierce, Rockford, IL) per well was added to the production plates to release protein from the cells. The production plates were diluted 100-fold in PBST (PBS containing 0.125% Tween-20), and BLA activity was measured as above.

Binding to CEA (carcinoembryonic antigen, Biodesign Intl., Saco, Maine) was measured using the following procedure: 96-well plates were coated with 100 ul per well of 5 ug/ml of CEA in 50 mM carbonate buffer pH 9.6 overnight. The plates were washed with PBST and blocked for 1-2 hours with 300 ul of casein (Pierce, Rockford, IL). 100 ul of sample from the production plate diluted 100-1000 fold was added to the CEA coated plate and the plates were incubated for 2 h at room temperature. Subsequently, the plates were washed four times with PBST and 200 ul nitrocefin assay buffer was added, and the BLA activity was measured as described above. CEA binding was measured in 50 mM phosphate buffer pH 6.5 and in a separate experiment in 50 mM phosphate buffer pH 7.4.

The BLA activity that was determined by the CEA-binding assay at pHs of 6.5 and 7.4, and the total BLA activity found in the lysate plates were compared and variants were identified which showed good binding to CEA at pH 6.5 but significantly weaker binding at pH 6.5. A comparison of the binding at pH6.5 versus pH 7.4 is shown in Figure 9.

Winners were confirmed by culturing them in 5 ml of LB medium containing 5 mg/l chloramphenicol and 0.1 mg/l cefotaxime (Sigma) for 2 days at 37 C. Subsequently, the cultures were centrifuged and the pellet was suspended in 375 ul of BPER reagent to release the fusion protein. BLA activity was determined as above. One unit of activity was defined as the amount of BLA that leads to an absorbance increase of one mOD per minute. The samples were diluted based on their total content of BLA activity and the CEA-binding assay was performed as described above but adding various sample dilutions to each well.

Binding curves for each sample that reflect the affinity of the variants to CEA can be obtained. Figure 11 shows CEA-binding curves measured at pH 7.4 and pH 6.5 for several variants of interest. All 5 variants show increased pH-dependence of CEA binding. Whereas, the parent NA06.6 binds only slightly better at pH 6.5 compared to pH 7.4, some of the variants show much stronger binding to CEA at pH 6.5 compared to pH 7.4. Variant NA08.15 which shows very weak binding to CEA at pH 7.4 but significant binding at pH 6.5; the variant was designated CAB1.4.

Table 5, below, shows the mutations in variants with the greatest binding improvement:

Table 5:

Clone	**Mutations**
NA08.1	W50H, Y166A
NA08.3	S190D, S226D
NA08.4	S190D, T100D
NA08.9	Y166A
NA08.12	T102H, Y166A, S226D
NA08.13	Q65H, S184D, S226D
NA08.14	P101D
NA08.15	S184D, S226D
NA08.17	S184D, W50H

Clone	Mutations
NA08.24	T102D, S226D
NA08.45	T102D, Y166A
NA08.51	P104H, Y166A
NA08.64	Q65D, Y166A

Example 3: Mutagenesis of CAB1.4 yielding CAB1.6

The codon for position T100 in the CDR3 of the heavy chain of CAB1.4 was subjected to saturation mutagenesis. For site saturation mutagenesis, complimentary oligos:

ME 239 F: ATTATTGTAATGAGGGGNNSCCGACTGGGCCGTACTA
ME 239 R: TAGTACGGCCCAGTCGGSNNCCCCTCATTACAATAAT,

were designed so a degenerate codon (NNS) would correspond with T100, flanked on either side by 17 base pairs of homology with CAB1.4. The oligo pair was used to carry out a QuickChange (Stratagene) reaction using CAB1.4 DNA as the template according to the manufacturers suggested protocol. After PCR cycling, the reaction mixture was digested with DpnI, and 1ul was used to transform 50ul of Invitrogen TOP10 electrocompetent cells. The transformation was plated on LA + 5ppm CMP + 0.1ppm CTX to select for clones that carry the selective marker and still produce active BLA after mutagenesis. Plates were then used to pick clones for screening. After screening, clone ME184.1 (=CAB1.6) that had a T100L mutation (ACT-CTC) was chosen for further optimization.

Example 4: Mutagenesis of CAB1.6 yielding SW149.5

Ten individual site saturation mini-libraries were created for 10 amino acid residues of the H3 CDR (G99, P101-Y109) of CAB1.6 molecule using plasmid

pME184.1 as a template with regular QuikChange mutagenesis protocol (Stratagene). After screening for improved affinity, clone pSW129.5 from mini-library SW129 and clone pSW134.1 from mini-library SW134 were isolated. Clone pSW129.5 recruited the T102L mutation from primers ME270F and ME270R, as shown below. Clone pSW134.1 recruited the F107N mutation from primers ME275F and ME275R, as shown below. Clone pSW129.5 was used as a template for further mutagenesis and to isolate clone pSW149.5 as described below.

Several mutations at positions P104 and Y105 were also identified in this screen. To combine those mutations as well as the F107N mutation of clone pSW134.1 into pSW129.5 backbone, a limited randomized library was created with primers SW133F and SW133R using pSW129.5 as a template. Subsequently, clone pSW149.5 was selected based on improved expression and affinity.

The following primers were used, as described above:

ME270F	GTAATGAGGGGCTGCCGNNSGGGCCGTACTACTTTGA
ME270R	TCAAAGTAGTACGGCCCSNNCGGCAGCCCCTCATTAC
ME275F	CGACTGGGCCGTACTACNNSGACTACTGGGGCCAAGG
ME275R	CCTTGGCCCCAGTAGTCSNNGTAGTACGGCCCAGTCG
SW133F	GAGGGGCTCCCGCTCGGGRVCNTTTACAACGACTACTGGGGCCAAGG
SW133R	CCTTGGCCCCAGTAGTCGTTGTAAANGBYCCCGAGCGGGAGCCCCTC

Example 5: Mutagenesis of SW149.5 yielding CAB1.7

Limited randomization of several amino acid residues of H2, L1 and L2 CDRs was achieved employing several degenerate primers. Residues targeted for limited randomization were: D57, T58, P62 and Q65 in the H2 CDR; S163, S165 and S166 in the L1 CDR and S186 and S190 in the L2 CDR. Screening of these variants allowed identification of positions in the protein likely to further improve its affinity for CEA. Library SW155 was created using primers SW134FP, SW135FP, SW136FP, SW137FP and SW138FP using the QuikChange multisite mutagenesis kit (Stratagene) as recommended by the manufacturer. The resulting library was screened and the best

variant, clone pSW155.17 was selected as it showed significantly improved binding to CEA; the clone was designated CAB1.7.

The following primers were used to generate library SW155:

SW134FP	[Phosp]CTTCTGGCTTCAACATTACCGACTCCTATATGCACTG
SW135FP	[Phosp]GCCTGGAGTGGATTGGATTTATTGATCCTGAGAATG
SW136FP	[Phosp]GATCCTGAGAATGGTSWTRCTGAATATGCCCBGAAGTTCRNCGGCAAGGCCACTTTTAC
SW137FP	[Phosp]CTGCAGTGCCAGCTCADCTGTAYMTDCCATGCACTGGTTCCAGC
SW138FP	[Phosp]CGTGATTTATGATACARVCAACCTGGCTRSTGGAGTCCCTGCTCGCTTC

Example 6: Generation of CAB1.6i and C AB1.7i

Figure 12 shows the development of CAB1.6i and CAB1.7i and demonstrates the incorporation of mutations in the process.

In order to compare the target-binding properties of various CAB1 variants, we grew 5 ml cultures of TOP10F' containing the corresponding expression plasmids, as provided above for 3 days at 25 C in LB medium containing 5 mg/l chloramphenicol. The cultures were centrifuged, and the resulting supernatant was discarded. The cell pellets were resuspended in 500 µL of B-PER reagent. This was incubated for 30 minutes. Lactamase concentration in each sample was determined using nitrocephin as substrate, as provided above.

Binding of the samples to microtiter plates coated with CEA was studied in 50 mM phosphate buffer at pH 6.5 and pH 7.4, as provided above. Binding curves are shown in Figure 13.

In a similar experiment, binding of variants to LS174T cells was measured. LS174T cells were inoculated in 96 well polystyrene plate at $1x10^5$ cells/well in a medium containing 70% DMEM, 30% F12, non-essential amino acids, L-Glut, and Sodium Pyruvate (all from Mediatech). The plate was incubated at 37°C in a humidified CO2 incubator for 20 hours. The cells were then fixed with 4% formaldehyde in PBS (Polysciences, Warrington, PA). The plate was washed with PBST, and 1mg/ml NaBH4 (Sigma) was added into each well to quench any reactive group. Then the plate was

washed again with PBST. Binding of CAB molecules was continued the same way as binding to CEA antigen.

Figure 14 shows binding curves for CAB1.2, CAB1.4, CAB1.6, and CAB1.6 to LS174T cells. CAB1.7 has a binding affinity at pH6.5 that closely resembles the binding curve of CAB1.2 at the same pH. In contrast, the binding curves at pH 7.4 show marked differences. At pH 7.4, CAB1.7 shows significantly weaker binding to tumor cells as compared to CAB1.2. Surprisingly, CAB1.7 binding curves reach saturation levels that are also pH-dependent. This suggests, that at saturation, more molecules of CAB1.7 can bind to tumor cells at pH6.5 as compared to pH7.4.

Example 7: Epitope removal of BLA

The i-mune assay was performed on the sequence for beta-lactamase as described (US Pat. Appln. Ser. No. 09/060,872, filed 4/15/98). Human population-based identification of CD4+ T cell peptide epitope determinants. (Journal of Immunological Methods, 281:95-108). Sixty-nine community donor peripheral blood cell samples were used. Four CD4+ T cell epitopes were identified. For each epitope peptide sequence, critical residue testing was performed. Critical residue testing included both an alanine scan of the peptide sequences, as well as specific amino acid modifications guided by functional and structural constraints. Peptide epitope sequences that reduced the level of proliferation to background levels were chosen and incorporated into a DNA construct of the beta-lactamase enzyme sequence. Modified enzyme protein variants were expressed and purified, then tested for their ability to induce cellular proliferation using human peripheral blood cells in vitro. The variant that induced the lowest level of cellular proliferation in vitro was selected for inclusion in CAB1.6 and CAB1.7.

Example 8: Construction of CAB1.6i and CAB1.7i

BLA genes in plasmids pME184.1 (CAB1.6) and pSW155.17 (CAB1.7) were mutated in order to introduce the de-immunized BLA (=BLAi) gene containing epitope-removing K265A and S568A mutations as described below. Using primers HR016F and

HR017F with the QuikChange Multisite mutagenesis kit (Stratagene) as recommended by the manufacturer, the two mutations were incorporated into plasmid pME184.1 (CAB1.6) resulting in plasmid pSW175.3 (CAB1.6i). For construction of plasmid pSW169.3 (CAB1.7i), a 0.9-kb NruI fragment of the BLA gene in plasmid pSW155.17 was exchanged with another 0.9-kb NruI fragment from plasmid pCD1.1which contains both mutations.

The following primers were used:

HR016F	[Phosp]GATTACCCCGCTGATGGCGGCCCAGTCTGTTCCAG
HR017F	[Phosp]CTACTGGCGGGTTTGGCGCGTACGTGGCCTTTATTCCTG

Having described the preferred embodiments of the present invention, it will appear to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations that is not specifically disclosed herein. The terms and expressions which have been employed are used as

terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

ABSTRACT

The present invention relates to CAB molecules, ADEPT constructs directed against CEA, and their use in diagnosis and therapy.

H1CDR (26)GFNIKDSYMH(35)

H2CDR (50)WIDPENGDTEYAPKFQ(65)

H3CDR (99)GTPTGPYYFDY(109)

L1CDR (159)SASSSVSYMH(168)

L2CDR (184)STSNLAS(190)

L3CDR (223)QQRSSYPLT(231)

Fig. 1. Amino acid sequences of CAB1 CDRs

SEQ ID NO: 1

```
  1  QVKLQQSGAE LVRSGTSVKL SCTASGFNIK DSYMHWLRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGT
101  PTGPYYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIMSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL WIYSTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRSSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 2A Amino acid sequence of CAB1 protein

SEQ ID NO: 2

```
  1 TPVSEKQLAE VVANTITPLM KAQSVPGMAV AVIYQGKPHY YTFGKADIAA
 51 NKPVTPQTLF ELGSISKTFT GVLGGDAIAR GEISLDDAVT RYWPQLTGKQ
101 WQGIRMLDLA TYTAGGLPLQ VPDEVTDNAS LLRFYQNWQP QWKPGTTRLY
151 ANASIGLFGA LAVKPSGMPY EQAMTTRVLK PLKLDHTWIN VPKAEEAHYA
201 WGYRDGKAVR VSPGMLDAQA YGVKTNVQDM ANWVMANMAP ENVADASLKQ
251 GIALAQSRYW RIGSMYQGLG WEMLNWPVEA NTVVETSFGN VALAPLPVAE
301 VNPPAPPVKA SWVHKTGSTG GFGSYVAFIP EKQIGIVMLA NTSYPNPARV
351 EAAYHILEAL Q
```

Fig. 2B Amino acid sequence of BLA protein

SEQ ID NO: 3

H1CDR (26)GFNIKDSYMH(35)

H2CDR (50)WIDPENGDTEYAPKFQ(65)

H3CDR (99)GLPTGPYYFDY(109)

L1CDR (159)SASSSVSYMH(168)

L2CDR (184)DTSNLAS(190)

L3CDR (223)QQRDSYPLT(231)

Fig. 3A Amino acid sequences of CAB1.6 CDRs

H1CDR (26)GFNIKDSYMH(35)

H2CDR (50)WIDPENGDTEYAPKFQ(65)

H3CDR (99)GLPLGAIYNDY(109)

L1CDR (159)SASSAVYAMH(168)

L2CDR (184)DTSNLAS(190)

L3CDR (223)QQRDSYPLT(231)

Fig. 3B Amino acid sequences of CAB1.7 CDRs

```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGL
101  PTGPYYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL VIYDTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRDSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 4A Amino acid sequence of CAB1.6 protein

SEQ ID NO. 7

```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGL
101  PTGPYYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL VIYDTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRDSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMAAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGAYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 4B Amino acid sequence of CAB1.6i protein

SEQ ID NO: 8

```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQQ PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGL
101  PLGAIYNDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSAVYAMHWF QQKPGTSPKL VIYDTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRDSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
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551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 5A Amino acid sequence of CAB1.7 protein

SEQ ID NO: 9

```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGL
101  PLGAIYNDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSAVYAMHWF QQKPGTSPKL VIYDTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRDSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMAAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGAYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 5B Amino acid sequence of CAB1.7i protein

SEQ ID NO: 10

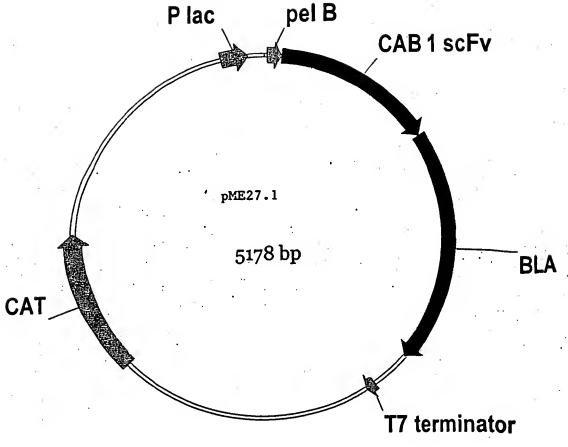

Figure 6A

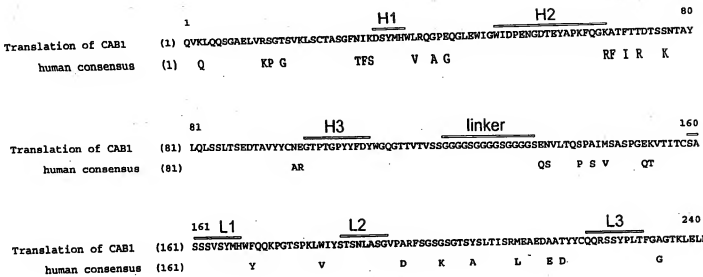
H1
H2
Translation of CAB1 (1) QVKLQQSGAELVRSGTSVKLSCTASGFNIKDSYMHWLRQGPEQGLEWIGWIDPENGDTEYAPKFQGKATFTTDTSSNTAY
human consensus (1) Q KP G TFS V A G RF I R K
H3
linker
Translation of CAB1 (81) LQLSSLTSEDTAVYYCNEGTPTGPYYFDYWGQGTTVTVSSGGGGSGGGGSGGGGSENVLTQSPAIMSASPGEKVTITCSA
human consensus (81) AR QS P S V QT
L1
L2
L3
Translation of CAB1 (161) SSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPLTFGAGTKLELKRAA
human consensus (161) Y V D K A L E D G

Figure 6B

aggaattatcatatgaaatacctgctgccgaccgctgctgctggtctgctgctcctcgctgcccagccggccatggcccaggtgaaactgcagcagt
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Figure 6C

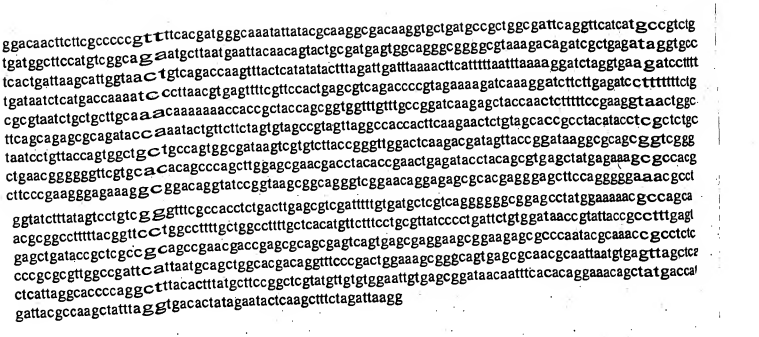
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Figure 6C

heavy chain:
qvklqqsgaelvrsgtsvklsctasgfnikdsymhwlrqgpeqglewigwidpengdteyapkfqgkatfttdtssntaylqlssltsedtavyycnegtptgpyyfdywgqgttvtvss

linker:
ggggsggggsggggs

light chain:
envltqspaimsaspgekvtitcsasssvsymhwfqqkpgtspklwiystsnlasgvparfsgsgsgtsysltisrmeaedaatyycqqrssypltfgagtklelkraat

BLA:
pvsekqlaevvantitplmkaqsvpgmavaviyqgkphyytfgkadiaankpvtpqtlfelgsisktftgvlggdaiargeislddavtrywpqltgkqwqgirmldlatytagglplqvpdevtd
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Figure 6D

bound BLA activity (U)
18
16
14
12
10
8
6
4
2
0
1000
10000
1e+005
BLA activity added (units/mL)
NA05.1
NA05.2
NA05.3
NA05.4
NA05.5
NA05.6
NA05.8
NA05.9
NA05.10
NA05.12
NA05.15
wild type

Figure7A

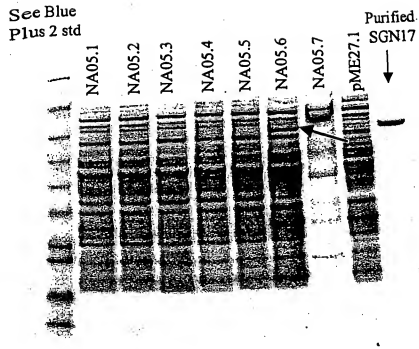

See Blue
Plus 2 std
NA05.1
NA05.2
NA05.3
NA05.4
NA05.5
NA05.6
NA05.7
pME27.1
Purified
SGN17

Figure 7B

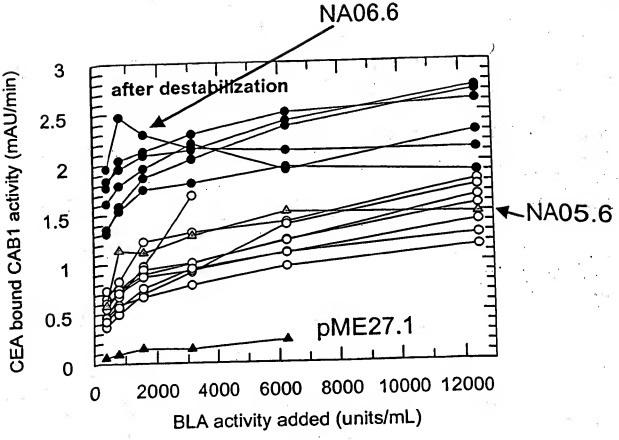

NA06.6
after destabilization
NA05.6
pME27.1
CEA bound CAB1 activity (mAU/min)
BLA activity added (units/mL)
0
0.5
1
1.5
2
2.5
3
0
2000
4000
6000
8000
10000
12000

Figure 7C

pos. h avy chain	number of observations	observ d frequencies of 5 most abundand amio acids in alignment of human sequenes														CAB1 sequence	CDR	mutated residues
1	291	E	0.616	Q	0.346	D	0.014	G	0.014	A	0.003	L	0.003	Q				
2	293	V	0.887	M	0.027	L	0.024	S	0.020	I	0.017	A	0.007	V				
3	291	Q	0.852	H	0.034	R	0.027	T	0.027	E	0.014	V	0.014	K				1
4	282	L	0.975	V	0.011	A	0.007	D	0.004	M	0.004			L				
5	276	V	0.645	Q	0.148	L	0.120	R	0.022	M	0.014	N	0.014	Q				
6	267	E	0.693	Q	0.263	A	0.022	D	0.011	G	0.007	R	0.004	Q				
7	265	S	0.951	W	0.019	X	0.015	T	0.008	A	0.004	N	0.004	S				
8	266	G	0.989	S	0.008	T	0.004							G				
9	274	G	0.624	A	0.193	P	0.164	S	0.011	E	0.004	H	0.004	A				
10	271	G	0.638	E	0.192	D	0.081	A	0.070	T	0.011	V	0.007	E				
11	270	L	0.681	V	0.270	F	0.030	S	0.019					L				
12	267	V	0.757	K	0.154	I	0.026	N	0.022	L	0.015	A	0.007	V				
13	247	K	0.474	Q	0.428	R	0.049	E	0.034	G	0.004	H	0.004	R				1
14	251	P	0.968	A	0.012	K	0.008	G	0.004	L	0.004	S	0.004	S				1
15	244	G	0.783	S	0.156	T	0.033	P	0.016	K	0.008	E	0.004	G				
16	243	G	0.488	E	0.131	Q	0.107	A	0.094	R	0.082	S	0.066	T				1
17	234	S	0.766	T	0.204	A	0.009	F	0.009	P	0.004	R	0.004	S				
18	244	L	0.812	V	0.155	M	0.008	A	0.004	E	0.004	F	0.004	V				
19	242	R	0.545	K	0.240	S	0.161	T	0.037	A	0.012	Q	0.004	K				
20	246	L	0.736	V	0.191	I	0.061	E	0.004	R	0.004	X	0.004	L				
21	218	S	0.729	T	0.234	G	0.009	I	0.009	A	0.005	D	0.005	S				
22	217	C	0.991	R	0.005	S	0.005							C				
23	231	A	0.558	K	0.203	T	0.117	E	0.048	V	0.022	I	0.013	T				
24	235	A	0.638	V	0.174	G	0.064	I	0.055	T	0.030	F	0.026	A				
25	226	S	0.951	Y	0.027	F	0.009	C	0.004	K	0.004	T	0.004	S				
26	225	G	0.956	E	0.013	A	0.009	D	0.009	S	0.009	V	0.004	G				
27	213	F	0.559	Y	0.164	G	0.150	D	0.080	S	0.019	L	0.014	F				
28	203	T	0.571	S	0.286	I	0.049	N	0.049	P	0.015	A	0.005	N				1
29	207	F	0.749	V	0.111	I	0.068	L	0.053	T	0.010	A	0.005	I				1
30	202	S	0.762	T	0.119	N	0.035	G	0.020	R	0.020	A	0.010	K				1
31	199	S	0.482	T	0.136	D	0.104	N	0.087	G	0.060	K	0.040	D		H1		
32	202	Y	0.535	S	0.144	N	0.083	A	0.069	D	0.031	G	0.030	S		H1		
33	197	A	0.269	Y	0.162	G	0.147	W	0.117	S	0.091	T	0.066	Y		H1		
34	200	M	0.520	I	0.210	W	0.070	A	0.055	Y	0.050	V	0.040	M		H1		
35	196	S	0.372	H	0.235	N	0.077	A	0.061	G	0.051	Y	0.046	H		H1		
35a	33	-	0.824	W	0.096	V	0.043	G	0.016	S	0.016	N	0.005			H2		
35b	27	-	0.856	N	0.064	G	0.037	S	0.032	A	0.005	R	0.005			H3		
36	192	W	0.990	M	0.005	T	0.005							W				
37	193	V	0.741	I	0.228	L	0.021	G	0.005	Q	0.005			L				1
38	190	R	0.989	P	0.005	V	0.005							R				
39	190	Q	0.979	T	0.011	G	0.005	R	0.005					Q				
40	191	A	0.634	P	0.199	S	0.073	M	0.052	G	0.010	V	0.010	G				1
41	187	P	0.914	S	0.043	T	0.021	A	0.005	L	0.005	Q	0.005	P				
42	187	G	0.925	S	0.064	P	0.005	R	0.005					E				1
43	186	K	0.683	Q	0.183	R	0.124	E	0.005	H	0.005			Q				
44	186	G	0.882	A	0.048	S	0.043	R	0.027					G				
45	186	L	0.978	P	0.022									L				
46	185	E	0.956	Q	0.039	V	0.005							E				
47	184	W	0.989	S	0.011									W				

Figure 8A

48	185	V	0.481	M	0.[illegible]	I	0.173	L	0.124								I						
49	185	G	0.600	S	0.216	A	0.162	E	0.005	L	0.005	T	[illegible]	0.005	G								
50	185	R	0.146	W	0.146	V	0.119	A	0.114	G	0.081	Y	0.081	W	H2								
51	185	I	0.822	T	0.081	R	0.027	V	0.022	K	0.016	M	0.011	I	H2								
52	184	S	0.250	Y	0.239	N	0.123	K	0.060	I	0.054	D	0.050	D	H2								
52a	141	-	0.230	P	0.180	Y	0.153	G	0.126	N	0.066	V	0.055	P	H2								
52b	34	-	0.814	K	0.115	R	0.060	G	0.005	Y	0.005				H2								
52c	22	-	0.880	T	0.044	V	0.033	S	0.022	A	0.011	G	0.005		H2								
53	184	S	0.228	D	0.163	Y	0.125	G	0.109	N	0.082	H	0.054	E	H2								
54	183	G	0.328	S	0.202	D	0.129	N	0.112	K	0.082	F	0.055	N	H2								
55	182	G	0.544	S	0.181	D	0.085	W	0.066	Y	0.060	N	0.020	G	H2								
56	182	S	0.231	D	0.182	N	0.147	T	0.143	Y	0.077	G	0.060	D	H2								
57	184	T	0.582	K	0.120	N	0.065	A	0.054	I	0.054	P	0.022	T	H2								
58	183	Y	0.322	N	0.216	D	0.139	R	0.060	H	0.055	T	0.038	E	H2								
59	184	Y	0.908	F	0.043	N	0.016	S	0.011	D	0.005	G	0.005	Y	H2								
60	183	A	0.579	N	0.153	S	0.104	T	0.055	R	0.044	G	0.027	A	H2								
61	184	D	0.277	P	0.239	Q	0.174	A	0.141	V	0.076	T	0.033	P	H2								
62	185	S	0.686	K	0.146	P	0.065	N	0.038	G	0.016	R	0.016	K	H2								
63	186	V	0.511	L	0.247	F	0.215	S	0.011	A	0.005	K	0.005	F	H2								
64	186	K	0.581	Q	0.274	R	0.054	N	0.032	E	0.022	T	0.022	Q	H2								
65	186	G	0.688	S	0.237	T	0.032	A	0.016	D	0.011	E	0.011	G	H2								
66	186	R	0.935	Q	0.054	H	0.005	I	0.005					K		1							
67	186	F	0.462	V	0.409	I	0.065	L	0.054	A	0.005	S	0.005	A		1							
68	186	T	0.914	I	0.038	A	0.016	S	0.011	K	0.005	N	0.005	T									
69	187	I	0.791	M	0.139	V	0.032	D	0.005	F	0.005	G	0.005	F		1							
70	187	S	0.684	T	0.214	N	0.070	L	0.032					T									
71	187	R	0.529	V	0.160	A	0.107	P	0.064	T	0.053	K	0.043	T		1							
72	186	D	0.902	N	0.071	K	0.016	E	0.011					D									
73	185	T	0.368	N	0.266	D	0.177	K	0.070	E	0.059	A	0.011	T									
74	186	S	0.946	A	0.048	L	0.005							S									
75	187	K	0.674	T	0.139	I	0.070	R	0.027	A	0.021	F	0.021	S		1							
76	187	N	0.701	S	0.251	K	0.027	R	0.011	T	0.005	Y	0.005	N									
77	187	T	0.615	Q	0.273	S	0.048	M	0.021	L	0.016	P	0.011	T									
78	186	L	0.364	A	0.273	F	0.235	V	0.096	I	0.005	M	0.005	A									
79	187	Y	0.638	S	0.239	F	0.059	V	0.048	H	0.005	M	0.005	Y									
80	187	L	0.782	M	0.207	N	0.005	-	0.005					L									
81	187	Q	0.529	E	0.205	K	0.122	R	0.032	T	0.032	N	0.027	Q									
82	194	M	0.497	L	0.421	W	0.051	V	0.015	I	0.010	-	0.005	L									
82a	195	N	0.442	S	0.291	R	0.077	T	0.066	D	0.053	G	0.020	S									
82b	194	S	0.795	N	0.082	R	0.051	G	0.026	T	0.021	A	0.010	S									
82c	197	L	0.701	V	0.234	M	0.041	G	0.010	A	0.005	D	0.005	L									
83	197	R	0.528	T	0.239	K	0.122	D	0.041	E	0.020	Q	0.015	T									
84	198	A	0.495	P	0.182	S	0.177	T	0.051	I	0.035	V	0.030	S									
85	198	E	0.591	A	0.172	D	0.126	S	0.051	V	0.045	G	0.015	E									
86	198	D	0.975	T	0.010	V	0.010	N	0.005					D									
87	198	T	0.929	S	0.035	G	0.010	M	0.010	A	0.005	Q	0.005	T									
88	198	A	0.939	G	0.040	P	0.005	T	0.005	V	0.005	Y	0.005	A									
89	198	V	0.768	L	0.066	M	0.056	T	0.045	I	0.040	F	0.010	V									
90	199	Y	0.980	F	0.010	A	0.005	I	0.005					Y									
91	199	Y	0.930	F	0.045	C	0.015	R	0.005	T	0.005			Y									
92	198	C	0.990	A	0.005	M	0.005							C									
93	198	A	0.838	T	0.076	V	0.061	H	0.005	K	0.005	N	0.005	N		1							
94	198	R	0.596	K	0.162	T	0.051	G	0.045	P	0.045	Q	0.025	E		1							
95	161	G	0.174	D	0.120	E	0.099	A	0.093	N	0.092	P	0.068	G									
96	159	P	0.168	R	0.130	G	0.112	L	0.062	V	0.062	Y	0.062	T	H3								
97	156	G	0.170	P	0.094	V	0.094	E	0.088	T	0.069	S	0.063	P	H3								
98	155	G	0.152	Y	0.101	L	0.095	D	0.087	V	0.076	S	0.063	T	H3								

Figure 8A

pos. light chain	number of observations	observed frequencies of 5 most abundand amio acids in alignment of human sequenes												CAB1 sequance	CDR	mutated residues
1	95	Q	0.589	S	0.158	N	0.095	H	0.074	D	0.053	F	0.021	E		1
2	139	S	0.446	Y	0.388	F	0.101	V	0.043	L	0.014	T	0.007	N		1
3	140	V	0.307	E	0.243	A	0.207	M	0.093	D	0.064	I	0.043	V		
4	140	L	0.971	V	0.029									L		
5	141	T	0.915	A	0.021	S	0.021	I	0.014	K	0.007	L	0.007	T		
6	140	Q	0.993	E	0.007									Q		
7	139	P	0.906	D	0.029	S	0.029	A	0.022	E	0.014			S		1
8	139	P	0.741	A	0.137	H	0.072	R	0.029	L	0.007	S	0.007	P		
9	139	S	0.964	A	0.014	V	0.014	R	0.007					A		1
10	0	-	1.000											I		1
11	138	V	0.790	A	0.138	L	0.058	M	0.014					M		1
12	139	S	0.978	F	0.007	T	0.007	E	0.004	Q	0.004			S		
13	138	V	0.406	G	0.348	A	0.138	E	0.087	L	0.014	D	0.007	A		
14	135	S	0.630	A	0.230	T	0.111	D	0.007	F	0.007	G	0.007	S		
15	135	P	0.881	L	0.089	A	0.022	S	0.007					P		
16	134	G	0.978	E	0.015	L	0.007							G		
17	133	Q	0.811	K	0.098	A	0.045	E	0.024	G	0.015	H	0.008	E		1
18	133	T	0.504	S	0.263	R	0.135	K	0.068	E	0.008	G	0.008	K		1
19	130	V	0.454	A	0.385	I	0.146	G	0.008	L	0.008			V		
20	128	T	0.531	R	0.188	S	0.148	K	0.047	I	0.031	M	0.016	T		
21	121	I	0.901	V	0.050	L	0.017	A	0.008	F	0.008	M	0.008	I		
22	120	S	0.492	T	0.475	A	0.008	G	0.008	I	0.008	N	0.008	T		
23	117	C	1.000											C		
24	112	S	0.536	T	0.259	G	0.089	A	0.045	Q	0.033	I	0.018	S	L1	
25	108	G	0.870	L	0.056	R	0.028	A	0.019	I	0.009	P	0.009	A	L1	
26	108	D	0.339	S	0.250	T	0.213	N	0.087	E	0.037	G	0.037	S	L1	
27	104	S	0.415	N	0.118	K	0.113	A	0.104	T	0.066	G	0.047	S	L1	
28	104	L	0.346	S	0.346	I	0.115	G	0.067	A	0.058	D	0.019	S	L1	
29	100	G	0.243	N	0.239	D	0.159	S	0.078	P	0.068	H	0.058	V	L1	
30	103	I	0.291	V	0.165	D	0.136	N	0.107	E	0.058	S	0.049	S	L1	
31	101	G	0.356	K	0.168	A	0.099	E	0.084	Q	0.084	D	0.069	Y	L1	
31a	54	-	0.438	S	0.167	G	0.104	N	0.083	Y	0.063	D	0.052	M	L1	
31b	49	-	0.495	N	0.227	Y	0.155	S	0.041	G	0.021	H	0.021	H	L1	
31c	23	-	0.760	N	0.134	S	0.031	K	0.021	D	0.012	E	0.010		L1	
31d	0	-	1.000												L1	
31e	0	-	1.000												L1	
31f	0	-	1.000												L1	
32	94	Y	0.515	S	0.134	F	0.093	A	0.072	T	0.052	H	0.041		L1	
33	97	V	0.680	A	0.186	I	0.082	Y	0.021	F	0.010	P	0.010		L1	
34	92	S	0.380	H	0.120	A	0.109	Y	0.098	N	0.076	Q	0.076		L1	
35	98	W	0.990	Y	0.010									W		
36	96	Y	0.844	F	0.073	H	0.073	W	0.010					F		1
37	95	Q	0.916	R	0.042	E	0.011	H	0.011	K	0.011	Y	0.011	Q		
38	94	Q	0.862	H	0.053	L	0.053	E	0.011	K	0.011	V	0.011	Q		
39	93	K	0.333	L	0.172	R	0.161	H	0.151	Q	0.086	V	0.043	K		
40	93	P	0.946	S	0.022	A	0.011	L	0.011	R	0.011			P		

Figure 8B

41	93	G	0.871	H	0.0	D	0.022	R	0.022	P	0.011		0.011	G					
42	92	Q	0.424	T	0.217	K	0.163	R	0.087	S	0.054	G	0.022	T					
43	92	A	0.717	S	0.174	G	0.065	T	0.022	L	0.011	V	0.011	S					
44	93	P	0.978	A	0.011	M	0.011							P					
45	92	K	0.391	V	0.315	R	0.109	L	0.065	T	0.065	A	0.033	K					
46	92	L	0.728	V	0.076	F	0.065	T	0.043	A	0.022	M	0.022	L					
47	91	V	0.484	L	0.374	I	0.077	M	0.055	N	0.011			W					1
48	91	I	0.791	V	0.110	M	0.077	L	0.011	S	0.011			I					
49	91	Y	0.769	F	0.110	R	0.066	H	0.022	D	0.011	I	0.011	Y					
50	89	D	0.303	E	0.210	Q	0.093	V	0.067	G	0.056	K	0.056	S	L2				
51	88	D	0.364	N	0.205	V	0.159	H	0.068	T	0.068	G	0.034	T	L2				
52	89	N	0.393	T	0.213	S	0.202	D	0.101	A	0.022	F	0.011	S	L2				
53	88	K	0.307	D	0.193	Q	0.182	N	0.080	E	0.057	S	0.057	N	L2				
54	88	R	0.875	X	0.068	K	0.034	L	0.011	W	0.011			L	L2				
55	86	P	0.851	G	0.080	S	0.023	A	0.011	H	0.011	R	0.011	A	L2				
56	85	S	0.837	D	0.081	P	0.023	A	0.012	L	0.012	T	0.012	S	L2				
57	86	G	0.920	E	0.034	S	0.011	T	0.011	W	0.011	-	0.011	G					
58	84	I	0.600	V	0.353	A	0.012	G	0.012	T	0.012	-	0.012	V					
59	84	P	0.847	S	0.106	A	0.012	L	0.012	V	0.012	-	0.012	P					
60	85	D	0.488	E	0.325	N	0.047	A	0.035	H	0.023	L	0.023	A					1
61	87	R	0.977	D	0.011	-	0.011							R					
62	88	F	0.943	I	0.034	L	0.011	R	0.011					F					
63	87	S	0.989	F	0.011									S					
64	87	G	0.885	A	0.069	S	0.023	V	0.023					G					
65	87	S	0.977	G	0.011	Y	0.011							S					
66	86	K	0.430	N	0.186	S	0.186	T	0.081	X	0.070	R	0.035	G					1
67	85	S	0.953	T	0.024	K	0.012	L	0.012					S					
68	85	G	0.859	S	0.071	A	0.035	D	0.024	Q	0.012			G					
69	85	N	0.434	T	0.318	A	0.129	D	0.036	G	0.024	K	0.024	T					
70	85	T	0.529	S	0.341	E	0.082	A	0.024	K	0.024			S					
71	85	A	0.847	R	0.082	V	0.059	S	0.012					Y					1
72	85	T	0.447	S	0.424	Y	0.082	A	0.035	I	0.012			S					
73	85	L	0.988	S	0.012									L					
74	85	T	0.706	A	0.165	G	0.106	I	0.012	L	0.012			T					
75	85	I	0.929	V	0.047	A	0.012	L	0.012					I					
76	85	S	0.718	T	0.200	N	0.035	I	0.024	G	0.012	R	0.012	S					
77	85	G	0.765	R	0.129	S	0.094	E	0.012					R					
78	85	L	0.588	V	0.224	T	0.106	A	0.071	G	0.012			M					1
79	85	Q	0.659	E	0.153	R	0.071	K	0.047	L	0.024	A	0.012	E					
80	85	A	0.459	S	0.235	T	0.200	V	0.047	P	0.035	N	0.012	A					
81	85	E	0.541	G	0.235	M	0.071	D	0.047	L	0.024	N	0.024	E					
82	85	D	0.964	N	0.024	E	0.012							D					
83	85	E	0.976	D	0.012	T	0.012							A					1
84	85	A	0.941	T	0.035	E	0.012	S	0.012					A					
85	85	D	0.859	E	0.082	H	0.024	A	0.012	I	0.012	M	0.012	T					1
86	85	Y	0.976	F	0.012	H	0.012							Y					
87	85	Y	0.894	F	0.106									Y					
88	85	C	0.988	H	0.012									C					
89	85	Q	0.482	A	0.153	S	0.141	G	0.094	C	0.059	N	0.035	Q	L3				
90	85	S	0.388	T	0.271	A	0.212	V	0.118	L	0.012			Q	L3				
91	85	W	0.576	Y	0.247	A	0.059	F	0.035	R	0.035	D	0.012	R	L3				
92	84	D	0.606	G	0.095	A	0.071	N	0.061	T	0.048	E	0.024	S	L3				
93	84	S	0.405	D	0.179	G	0.107	N	0.095	P	0.071	T	0.060	S	L3				
94	84	S	0.536	G	0.155	N	0.073	R	0.060	D	0.058	T	0.048	Y	L3				
95	82	S	0.265	L	0.253	G	0.108	N	0.096	T	0.084	A	0.036	P	L3				

Figure 8B

95a	60	-	0.268	S	0.183	D	0.159	N	0.110	T	0.073	Q	0.049	L	L3		
95b	40	-	0.512	A	0.098	G	0.098	H	0.085	E	0.049	R	0.037	T	L3		
95c	5	-	0.939	P	0.037	A	0.012	G	0.012						L3		
95d	1	-	0.988	G	0.012										L3		
95	0	-	1.000												L3		
95f	0	-	1.000												L3		
96	80	V	0.305	G	0.098	P	0.098	W	0.098	A	0.073	N	0.073		L3		
97	85	V	0.788	I	0.118	L	0.047	M	0.035	G	0.012						
98	86	F	0.988	V	0.012									F			
99	89	G	0.989	F	0.011									G			
100	89	G	0.831	T	0.124	A	0.022	S	0.022					A			1
101	89	G	1.000											G			
102	89	T	0.989	G	0.011									T			
103	88	K	0.739	N	0.091	R	0.068	Q	0.034	T	0.034	E	0.011	K			
104	87	L	0.667	V	0.322	Q	0.011							L			
105	87	T	0.954	S	0.023	I	0.011	L	0.011					E			1
106	85	V	0.988	T	0.012									L			1
106a	84	L	0.952	V	0.024	P	0.012	Q	0.012					K			1
107	78	G	0.782	S	0.103	R	0.090	C	0.013	L	0.013			R			1
108	46	Q	0.957	P	0.022	R	0.022							A			1
109	46	P	0.957	K	0.022	Q	0.022							A			1

Figure 8B

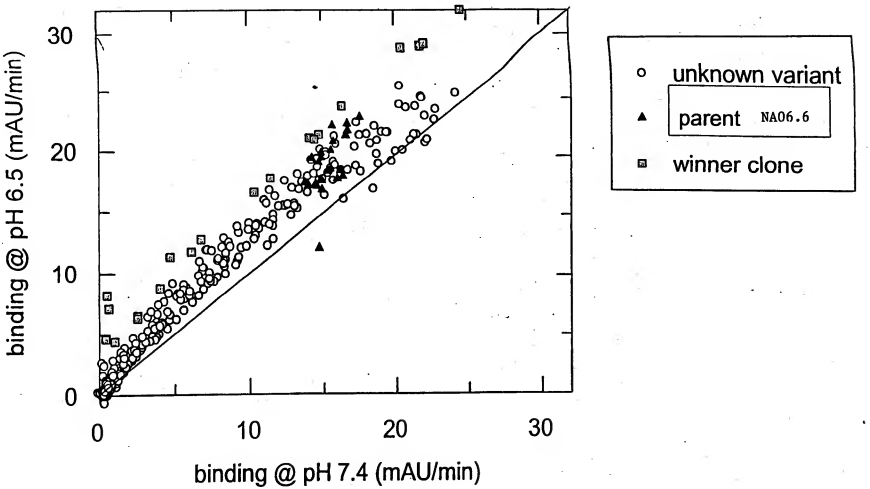
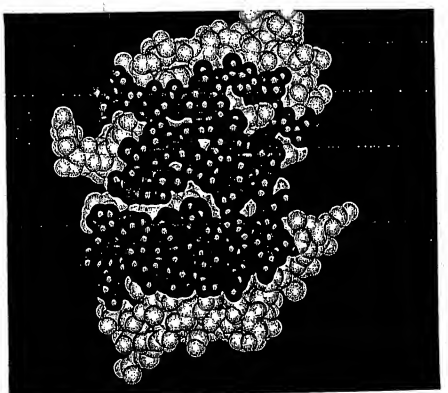

binding @ pH 6.5 (mAU/min)
30
20
10
0
0
10
20
30
binding @ pH 7.4 (mAU/min)
unknown variant
parent NA06.6
winner clone

Figure 9

CDRs in NA06.6



Residues chosen for mutagenesis in NA06.6

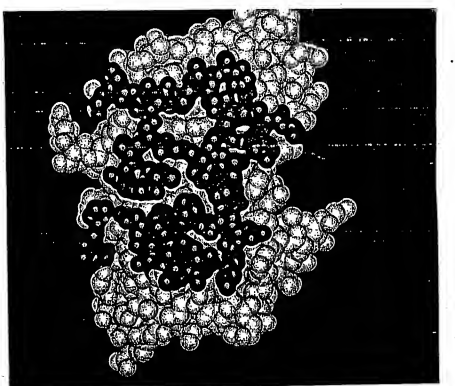


Figure 10

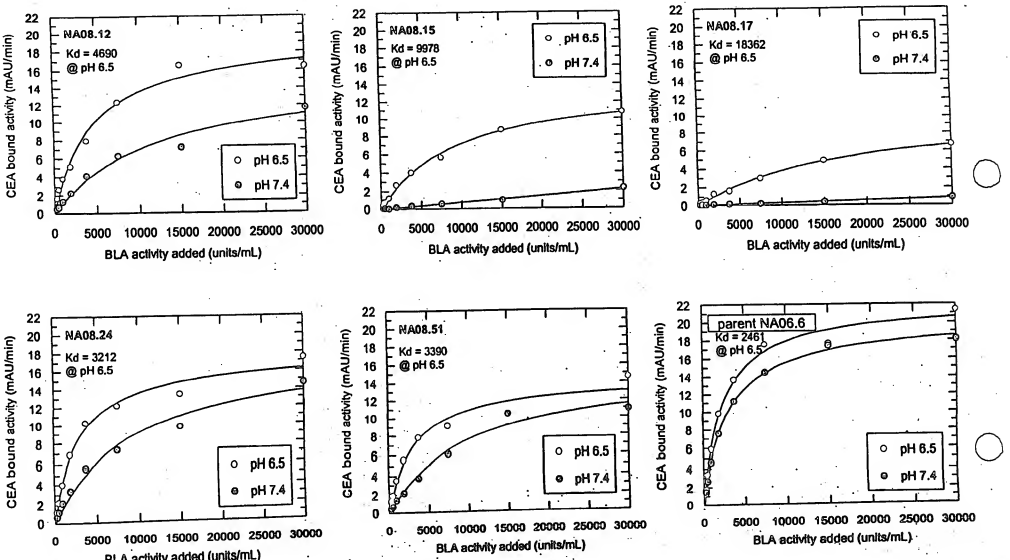

NA08.12
Kd = 4690
@ pH 6.5
pH 6.5
pH 7.4
CEA bound activity (mAU/min)
BLA activity added (units/mL)
NA08.15
Kd = 9978
@ pH 6.5
NA08.17
Kd = 18362
@ pH 6.5
NA08.24
Kd = 3212
@ pH 6.5
NA08.51
Kd = 3390
@ pH 6.5
parent NA06.6
Kd = 246
@ pH 6.5

Figure 11

Figure 12: CAB1 engineering - summary

protein	changes	comments
CAB1	parent	
CAB1.1(NA05.6)	R13K, T16G, W181V	increase stability
CAB1.2(NA06.6)	K3Q, L37V, M146V	increase stability
CAB1.4(NA08.15)	S184D, S226D	pH-dependent binding
CAB1.6	T100L	increased affinity
SW149.5	T102L, P104A, Y105I, F107N	increased affinity
CAB1.7	S163T, S165Y, Y166S	increased affinity
CAB1.7i	in BLA: K265A, S568A	remove T-cell epitopes

Figure 13: Binding of various CAB1 variants to immobilized CEA

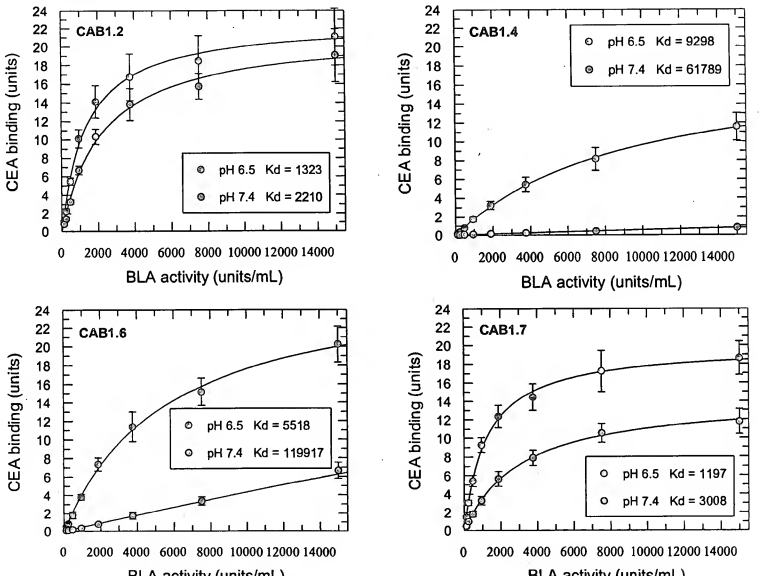
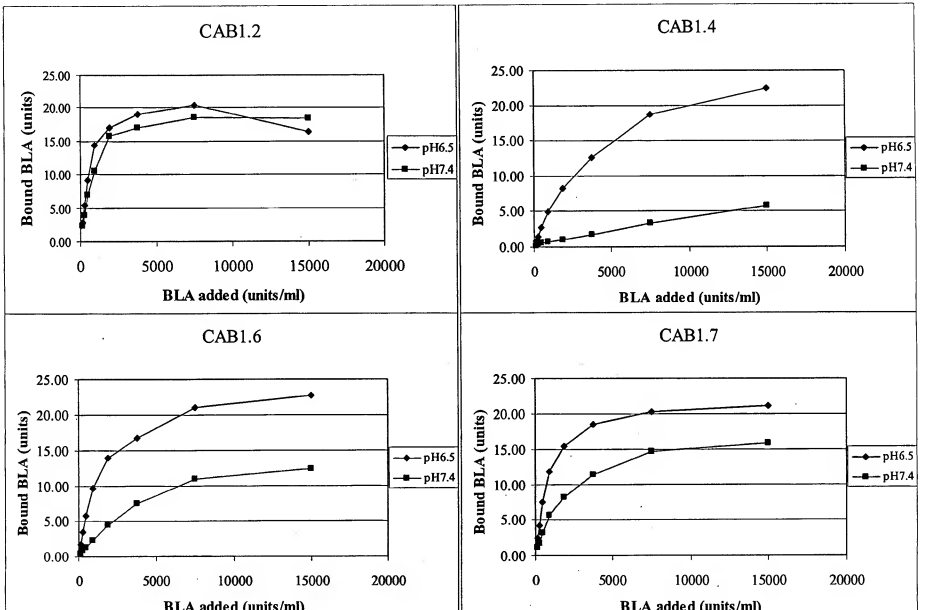


Figure 14: Binding of various CAB1 variants to LS174T cells



```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGL
101  PLGAIYNDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL VIYDTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRDSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 15A Amino acid sequence of SW149.5 protein

```
  1  QVKLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWLRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGT
101  PTGPYYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIMSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL VIYSTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRSSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 15B Amino acid sequence of CAB1.1 protein

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGACT
 301  CCGACTGGGC CGTACTACTT TGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATA
 551  GCACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGATCTAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15C Nucleotide sequence of CAB1.2 gene

```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGT
101  PTGPYYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL VIYSTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRSSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 15D Amino acid sequence of CAB1.2 protein

H1CDR (26)GFNIKDSYMH(35)

H2CDR (50)WIDPENGDTEYAPKFQ(65)

H3CDR (99)GTPTGPYYFDY(109)

L1CDR (159)SASSSVSYMH(168)

L2CDR (184)DTSNLAS(190)

L3CDR (223)QQRDSYPLT(231)

Fig. 15E Amino acid sequences of CAB1.4 CDRs

H1CDR (154)GGCTTCAACATTAAAGACTCCTATATGCAC(183)

H2CDR (226)TGGATTGATCCTGAGAATGGTGATACTGAATATGCCCCGAAGTTCCAG(273)

H3CDR (373)GGGACTCCGACTGGGCCGTACTACTTTGACTAC(405)

L1CDR (553)AGTGCCAGCTCAAGTGTAAGTTACATGCAC(582)

L2CDR (628)GATACATCCAACCTGGCTTCT(648)

L3CDR (745)CAGCAAAGAGATAGTTACCCACTCACG(771)

Fig. 15F Nucleotide sequence of CAB1.4 CDRs

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGACT
 301  CCGACTGGGC CGTACTACTT TGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATG
 551  ATACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGAGATAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15G. Nucleotide sequence of CAB1.4 gene

```
  1  QVQLQQSGAE LVKSGGSVKL SCTASGFNIK DSYMHWVRQG PEQGLEWIGW
 51  IDPENGDTEY APKFQGKATF TTDTSSNTAY LQLSSLTSED TAVYYCNEGT
101  PTGPYYFDYW GQGTTVTVSS GGGGSGGGGS GGGGSENVLT QSPAIVSASP
151  GEKVTITCSA SSSVSYMHWF QQKPGTSPKL VIYDTSNLAS GVPARFSGSG
201  SGTSYSLTIS RMEAEDAATY YCQQRDSYPL TFGAGTKLEL KRAATPVSEK
251  QLAEVVANTI TPLMKAQSVP GMAVAVIYQG KPHYYTFGKA DIAANKPVTP
301  QTLFELGSIS KTFTGVLGGD AIARGEISLD DAVTRYWPQL TGKQWQGIRM
351  LDLATYTAGG LPLQVPDEVT DNASLLRFYQ NWQPQWKPGT TRLYANASIG
401  LFGALAVKPS GMPYEQAMTT RVLKPLKLDH TWINVPKAEE AHYAWGYRDG
451  KAVRVSPGML DAQAYGVKTN VQDMANWVMA NMAPENVADA SLKQGIALAQ
501  SRYWRIGSMY QGLGWEMLNW PVEANTVVET SFGNVALAPL PVAEVNPPAP
551  PVKASWVHKT GSTGGFGSYV AFIPEKQIGI VMLANTSYPN PARVEAAYHI
601  LEALQ
```

Fig. 15H Amino acid sequence of CAB1.4 protein

H1CDR (154)GGCTTCAACATTAAAGACTCCTATATGCAC(183)

H2CDR (226)TGGATTGATCCTGAGAATGGTGATACTGAATATGCCCCGAAGTTCCAG(273)

H3CDR (373)GGGCTCCCGACTGGGCCGTACTACTTTGACTAC(405)

L1CDR (553)AGTGCCAGCTCAAGTGTAAGTTACATGCAC(582)

L2CDR (628)GATACATCCAACCTGGCTTCT(648)

L3CDR (745)CAGCAAAGAGATAGTTACCCACTCACG(771)

Fig. 15I Nucleotide sequences of CAB1.6 CDRs

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGCTC
 301  CCGACTGGGC CGTACTACTT TGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATG
 551  ATACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGAGATAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15J Nucleotide sequence of CAB1.6 gene

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGCTC
 301  CCGACTGGGC CGTACTACTT TGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATG
 551  ATACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGAGATAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGGCGGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CGCGTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15K Nucleotide sequence of CAB1.6i gene

H1CDR (154)GGCTTCAACATTAAAGACTCCTATATGCAC(183)

H2CDR (226)TGGATTGATCCTGAGAATGGTGATACTGAATATGCCCCGAAGTTCCAG(273)

H3CDR (373)GGGCTCCCGCTCGGGGCCATTTACAACGACTAC(405)

L1CDR (553)AGTGCCAGCTCAGCTGTATATGCCATGCAC(582)

L2CDR (628)GATACATCCAACCTGGCTTCT(648)

L3CDR (745)CAGCAAAGAGATAGTTACCCACTCACG(771)

Fig. 15L Nucleotide sequences of CAB1.7 CDRs

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGCTC
 301  CCGCTCGGGG CCATTTACAA CGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAGCTG TATATGCCAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATG
 551  ATACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGAGATAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15M Nucleotide sequence of CAB1.7 gene

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGCTC
 301  CCGCTCGGGG CCATTTACAA CGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAGCTG TATATGCCAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATG
 551  ATACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGAGATAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGGCGGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CGCGTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15N Nucleotide sequence of CAB1.7i gene

H1CDR (154) GGCTTCAACATTAAAGACTCCTATATGCAC (183)

H2CDR (226) TGGATTGATCCTGAGAATGGTGATACTGAATATGCCCCGAAGTTCCAG (273)

H3CDR (373) GGGACTCCGACTGGGCCGTACTACTTTGACTAC (405)

L1CDR (553) AGTGCCAGCTCAAGTGTAAGTTACATGCAC (582)

L2CDR (628) AGCACATCCAACCTGGCTTCT (648)

L3CDR (745) CAGCAAAGATCTAGTTACCCACTCACG (771)

Fig. 150 Nucleotide sequences of CAB1 CDRs

```
   1  CAGGTGAAAC TGCAGCAGTC TGGGGCAGAA CTTGTGAGGT CAGGGACCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGTT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGACT
 301  CCGACTGGGC CGTACTACTT TGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCATGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC TGGATTTATA
 551  GCACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGATCTAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15P Nucleotide sequence of CAB1 gene

H1CDR

(26)GFNIKDSYMH(35)

H2CDR (50)WIDPENGDTEYAPKFQ(65)

H3CDR (99)GLPLGAIYNDY(109)

L1CDR (159)SASSSVSYMH(168)

L2CDR (184)DTSNLAS(190)

L3CDR (223)QQRDSYPLT(231)

Fig. 15Q Amino acid sequences of SW149.5 CDRs

H1CDR (154) GGCTTCAACATTAAAGACTCCTATATGCAC (183)

H2CDR (226) TGGATTGATCCTGAGAATGGTGATACTGAATATGCCCCGAAGTTCCAG (273)

H3CDR (373) GGGCTCCCGCTCGGGGCCATTTACAACGACTAC (405)

L1CDR (553) AGTGCCAGCTCAAGTGTAAGTTACATGCAC (582)

L2CDR (628) GATACATCCAACCTGGCTTCT (648)

L3CDR (745) CAGCAAAGAGATAGTTACCCACTCACG (771)

Fig. 15R Nucleotide sequences of SW149.5 CDRs

```
   1  CAGGTGCAGC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGGT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGCTC
 301  CCGCTCGGGG CCATTTACAA CGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCGTGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATG
 551  ATACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGAGATAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
```

Fig. 15S Nucleotide sequence of SW149.5 gene

```
   1  ACACCGGTGT CAGAAAAACA GCTGGCGGAG GTGGTCGCGA ATACGATTAC
  51  CCCGCTGATG AAAGCCCAGT CTGTTCCAGG CATGGCGGTG GCCGTTATTT
 101  ATCAGGGAAA ACCGCACTAT TACACATTTG GCAAGGCCGA TATCGCGGCG
 151  AATAAACCCG TTACGCCTCA GACCCTGTTC GAGCTGGGTT CTATAAGTAA
 201  AACCTTCACC GGCGTTTTAG GTGGGGATGC CATTGCTCGC GGTGAAATTT
 251  CGCTGGACGA TGCGGTGACC AGATACTGGC CACAGCTGAC GGGCAAGCAG
 301  TGGCAGGGTA TTCGTATGCT GGATCTCGCC ACCTACACCG CTGGCGGCCT
 351  GCCGCTACAG GTACCGGATG AGGTCACGGA TAACGCCTCC CTGCTGCGCT
 401  TTTATCAAAA CTGGCAGCCG CAGTGGAAGC CTGGCACAAC GCGTCTTTAC
 451  GCCAACGCCA GCATCGGTCT TTTTGGTGCG CTGGCGGTCA AACCTTCTGG
 501  CATGCCCTAT GAGCAGGCCA TGACGACGCG GGTCCTTAAG CCGCTCAAGC
 551  TGGACCATAC CTGGATTAAC GTGCCGAAAG CGGAAGAGGC GCATTACGCC
 601  TGGGGCTATC GTGACGGTAA AGCGGTGCGC GTTTCGCCGG GTATGCTGGA
 651  TGCACAAGCC TATGGCGTGA AAACCAACGT GCAGGATATG GCGAACTGGG
 701  TCATGGCAAA CATGGCGCCG GAGAACGTTG CTGATGCCTC ACTTAAGCAG
 751  GGCATCGCGC TGGCGCAGTC GCGCTACTGG CGTATCGGGT CAATGTATCA
 801  GGGTCTGGGC TGGGAGATGC TCAACTGGCC CGTGGAGGCC AACACGGTGG
 851  TCGAGACGAG TTTTGGTAAT GTAGCACTGG CGCCGTTGCC CGTGGCAGAA
 901  GTGAATCCAC CGGCTCCCCC GGTCAAAGCG TCCTGGGTCC ATAAAACGGG
 951  CTCTACTGGC GGGTTTGGCA GCTACGTGGC CTTTATTCCT GAAAAGCAGA
1001  TCGGTATTGT GATGCTCGCG AATACAAGCT ATCCGAACCC GGCACGCGTT
1051  GAGGCGGCAT ACCATATCCT CGAGGCGCTA CAG
```

Fig. 15T Nucleotide sequence of BLA gene

```
   1  CAGGTGAAAC TGCAGCAGTC TGGGGCAGAA CTTGTGAAAT CAGGGGGCTC
  51  AGTCAAGTTG TCCTGCACAG CTTCTGGCTT CAACATTAAA GACTCCTATA
 101  TGCACTGGTT GAGGCAGGGG CCTGAACAGG GCCTGGAGTG GATTGGATGG
 151  ATTGATCCTG AGAATGGTGA TACTGAATAT GCCCCGAAGT TCCAGGGCAA
 201  GGCCACTTTT ACTACAGACA CATCCTCCAA CACAGCCTAC CTGCAGCTCA
 251  GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTATTGTAA TGAGGGGACT
 301  CCGACTGGGC CGTACTACTT TGACTACTGG GGCCAAGGGA CCACGGTCAC
 351  CGTCTCCTCA GGTGGAGGCG GTTCAGGCGG AGGTGGCTCT GGCGGTGGCG
 401  GATCAGAAAA TGTGCTCACC CAGTCTCCAG CAATCATGTC TGCATCTCCA
 451  GGGGAGAAGG TCACCATAAC CTGCAGTGCC AGCTCAAGTG TAAGTTACAT
 501  GCACTGGTTC CAGCAGAAGC CAGGCACTTC TCCCAAACTC GTGATTTATA
 551  GCACATCCAA CCTGGCTTCT GGAGTCCCTG CTCGCTTCAG TGGCAGTGGA
 601  TCTGGGACCT CTTACTCTCT CACAATCAGC CGAATGGAGG CTGAAGATGC
 651  TGCCACTTAT TACTGCCAGC AAAGATCTAG TTACCCACTC ACGTTCGGTG
 701  CTGGCACCAA GCTGGAGCTG AAACGGGCGG CCACACCGGT GTCAGAAAAA
 751  CAGCTGGCGG AGGTGGTCGC GAATACGATT ACCCCGCTGA TGAAAGCCCA
 801  GTCTGTTCCA GGCATGGCGG TGGCCGTTAT TTATCAGGGA AAACCGCACT
 851  ATTACACATT TGGCAAGGCC GATATCGCGG CGAATAAACC CGTTACGCCT
 901  CAGACCCTGT TCGAGCTGGG TTCTATAAGT AAAACCTTCA CCGGCGTTTT
 951  AGGTGGGGAT GCCATTGCTC GCGGTGAAAT TTCGCTGGAC GATGCGGTGA
1001  CCAGATACTG GCCACAGCTG ACGGGCAAGC AGTGGCAGGG TATTCGTATG
1051  CTGGATCTCG CCACCTACAC CGCTGGCGGC CTGCCGCTAC AGGTACCGGA
1101  TGAGGTCACG GATAACGCCT CCCTGCTGCG CTTTTATCAA AACTGGCAGC
1151  CGCAGTGGAA GCCTGGCACA ACGCGTCTTT ACGCCAACGC CAGCATCGGT
1201  CTTTTTGGTG CGCTGGCGGT CAAACCTTCT GGCATGCCCT ATGAGCAGGC
1251  CATGACGACG CGGGTCCTTA AGCCGCTCAA GCTGGACCAT ACCTGGATTA
1301  ACGTGCCGAA AGCGGAAGAG GCGCATTACG CCTGGGGCTA TCGTGACGGT
1351  AAAGCGGTGC GCGTTTCGCC GGGTATGCTG GATGCACAAG CCTATGGCGT
1401  GAAAACCAAC GTGCAGGATA TGGCGAACTG GGTCATGGCA AACATGGCGC
1451  CGGAGAACGT TGCTGATGCC TCACTTAAGC AGGGCATCGC GCTGGCGCAG
1501  TCGCGCTACT GGCGTATCGG GTCAATGTAT CAGGGTCTGG GCTGGGAGAT
1551  GCTCAACTGG CCCGTGGAGG CCAACACGGT GGTCGAGACG AGTTTTGGTA
1601  ATGTAGCACT GGCGCCGTTG CCCGTGGCAG AAGTGAATCC ACCGGCTCCC
1651  CCGGTCAAAG CGTCCTGGGT CCATAAAACG GGCTCTACTG GCGGGTTTGG
1701  CAGCTACGTG GCCTTTATTC CTGAAAAGCA GATCGGTATT GTGATGCTCG
1751  CGAATACAAG CTATCCGAAC CCGGCACGCG TTGAGGCGGC ATACCATATC
1801  CTCGAGGCGC TACAG
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Fig. 15U Nucleotide sequence of CAB1.1 gene